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MICROBIAL AND CHEMICAL STUDIES OF THE CLOVERBAR INDUSTRIAL LAGOONS

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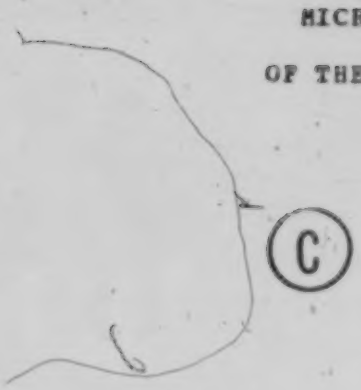

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"Microbial and Chemical Studies of
the Cloverbar Industrial Lagoons"

submitted by Phillip M. Fedorak in partial fulfilment of the requirements for the degree of Master of Science.

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Date September 8 1975.

ABSTRACT

The Cloverbar industrial lagoons receive an average daily flow of slightly over six million imperial gallons of wastewater. Approximately half of this volume is domestic waste and the remainder is material from three large packinghouses in Edmonton, Alberta. Lime added to the incoming wastes controls odors and regulates the pH of the lagoon contents. At present, one of the three anaerobic lagoons is not in use because of its thick grease cover, but the others are used in series to treat the incoming wastes. (The second lagoon now also has a thin grease cover.) The microbial, chemical and physical factors affecting the treatment efficiency of the three anaerobic lagoons were the object of this study.

Various methods of handling alkaline samples obtained for bacteriological counts and the effects of prereduced dilution buffer on the numbers of sulfate reducers detected in a wastewater sample were evaluated. Microbial and chemical analyses were carried out on samples taken from various locations within the system in order to evaluate the purification process. It was also hoped that this study would disclose operational changes which could be used to alter the rates of biochemical activities in the lagoons.

The findings indicated that alkaline samples taken for bacterial enumeration should be diluted in the field to

increase the survival of the microbes during transit. It was also observed that the number of sulfate reducers detected in a sample was nearly independent of the extent to which the dilution buffer was prereduced.

The addition of lime in the sewer line chemically controls hydrogen sulfide liberation, but does not significantly decrease the number of sulfate-reducing bacteria. Large numbers of sulfate reducers were found in the grease blankets indicating that (1) anaerobic conditions exist through essentially the entire depth of a lagoon and (2) the presence of such a cover probably does not aid odor control.

A carbon to nitrogen ratio of about 10:1 is theoretically ideal for bacterial growth and activity. However the ratios observed in the of the Cloverbar lagoons deviated from this value. The grease blankets had C:N ratios between 30:1 and 60:1 whereas the liquid in the final lagoon had ratios near 4:1. An attempt was made to adjust these by recycling part of the final effluent through the system. However, these efforts failed because an insufficient volume of effluent was recycled.

The amounts and proportions of various long chain fatty acids present in the influents, effluents and at different depths in the lagoons were also determined. The scum was found to contain concentrations of cis-9-octadecenoic acid

which are inhibitory to methane bacteria. Biohydrogenation was evident in the system and was most noticable in the scum blankets where, compared to the raw influent, the proportions of cis-9-octadecenoic acid decreases about 4 fold. The sludges of the three lagoons, have similiar concentrations of grease, but differ with regard to their proportions of fatty acids.

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ABBREVIATIONS USED

APHA ....American Public Health Association

BOD .....Biochemical Oxygen Demand

C12 .....dodecanoic acid

C14 .....tetradecanoic acid

C16 .....hexadecanoic acid

C16:1 ...cis-9-hexadecenoic acid

C18 .....octadecanoic acid

C18:1 ...cis-9-octadecenoic acid

C18:2 ...cis,cis-9,12-octadecadienoic acid

MPN .....Most Probable Number

ppm .....parts per million

SD .....Standard Deviation

w/v .....weight per volume

INTRODUCTION

The City of Edmonton's waste treatment facilities consist of the Main Sewage Treatment Plant, which provides full primary and secondary treatment for about 40 million imperial gallons of wastewater per day and the Cloverbar industrial lagoons which handle, mainly, high strength packinghouse wastes. The importance of the latter installation is especially evident at times when the packinghouse wastes are diverted through the treatment plant. Table 1 shows the average loading and gas production at the main plant during November, 1973, and January, 1974. During November, the plant was operating under normal load, whereas, during January, 1974, it was receiving packinghouse wastes because of a break in the sewer line to the lagoons. While the average daily flow increased only very slightly, the influent BOD increased 29%, and both the suspended solids and the volume of digester gas produced increased 21%.

The Cloverbar industrial lagoons are located on the south bank of the North Saskatchewan River, downstream from Edmonton, and about three miles northeast of the city limits as shown in Figure 1.

The lagoon system, constructed in 1964, originally consisted of three anaerobic lagoons and two storage ponds. In 1972, a third storage pond was added. The bottoms and

Table 1. Effect of packinghouse wastes on selected operating parameters of the City of Edmonton Main Sewage Treatment Plant

	Influent[a]			
	Flow (megagal)	BOD (lb)	Suspended Solids (lb)	Digester gas produced[a] (1000 ft^3)
November, 1973 (normal load)	39.8	91,074	90,735	646
January, 1974 (normal load plus packinghouse wastes)	40.5	128,661	114,229	823
Percentage increase due to packinghouse wastes	1.7	29	21	21

[a]daily average

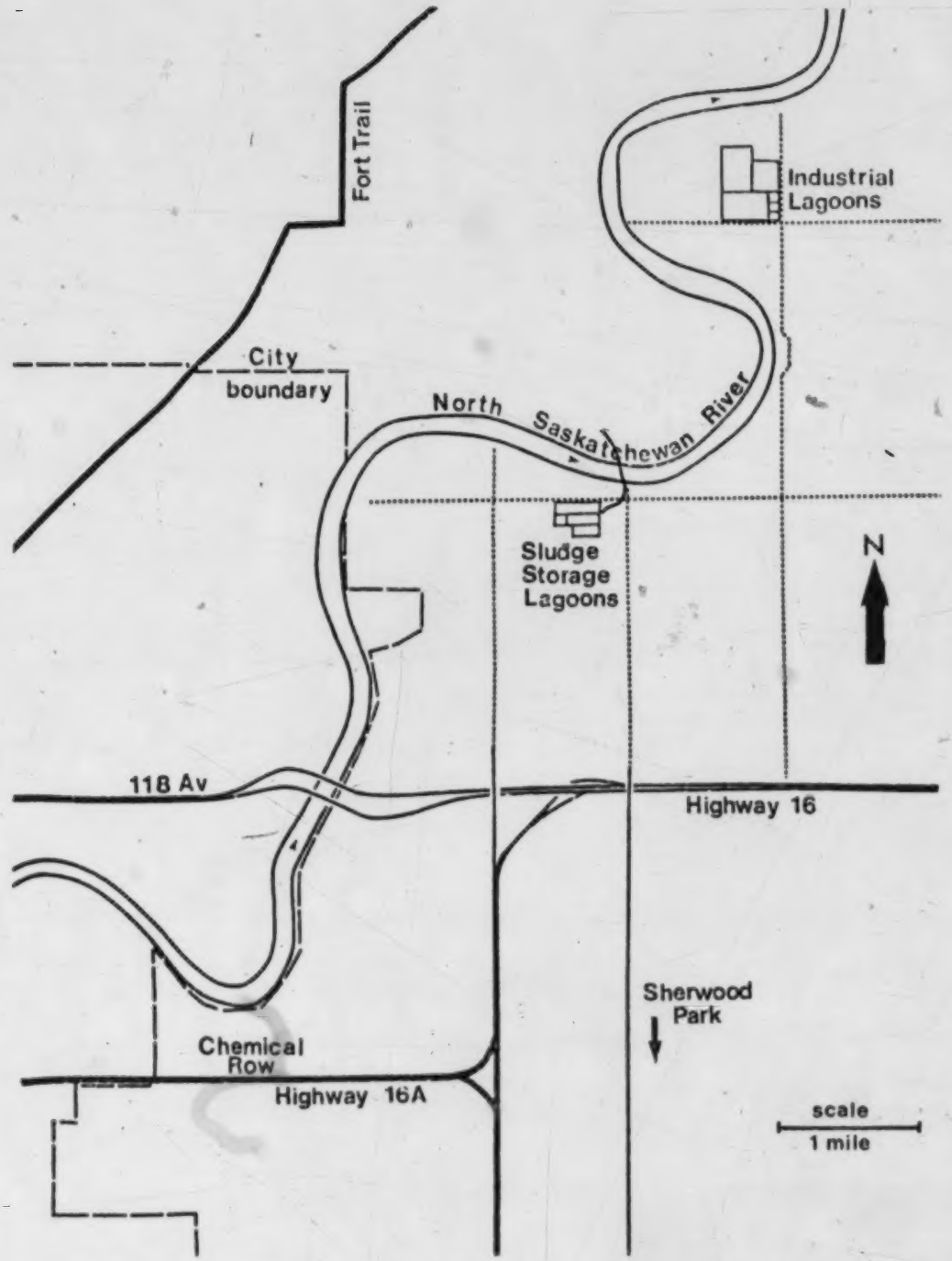


Figure 1. Locations of the Cloverbar industrial lagoons and the City of Edmonton sludge storage lagoons.

sides of the three anaerobic lagoons are lined with approximately three inches of asphalt. Each lagoon is just under two acres in area and is operated at a depth of 20 feet. This is a volume of 1.3 million cubic feet or 8.1 million imperial gallons in each lagoon. The storage ponds are all 20 feet deep and have areas of 20, 52, and 40 acres. They are filled only during the winter months, (November to May) when it would be unwise to burden the ice-covered, low-flowing, North Saskatchewan River with any extra organic load. During spring break-up, while the river is at its peak flow, these ponds are drained over a three week period. During the summer, the effluents from the anaerobic cells stream through the empty storage lagoons, en route to the river.

The object of this study was to determine and attempt to control the factors affecting the operation and efficiency of the anaerobic lagoons. The system was originally designed to handle wastes from three large packinghouses located in northeast Edmonton. At that time, their combined waste flow was about 2.8 million imperial gallons/day; giving a theoretical detention time of nearly 3 days in each cell (Stanley, 1966). Design criteria and operation experiences until 1970 are discussed by Stanley (1966) and Bouthillier and Brown (1971).

The stablization process involves passing the wastewaters through two anaerobic lagoons in series. From

the initial start-up (winter 1964-65) to October, 1969, cells 1 and 2 were used in series leaving cell 3 empty. However, in 1969, the flow pattern had to be altered due to an accumulation of grease in cell 1. Since that time, cells 2 and 3 have been used in series, and cell 1 has received wastes only for brief periods during the summer of 1972 and the fall of 1973.

Since this study began in May 1973, high strength wastes, from the packing plants, and domestic wastes from Oliver, Beverly, and Sherwood Park have been treated in the Cloverbar industrial lagoons. During the summer of 1974, the system also received supernatant from the City of Edmonton sludge storage lagoons which are located about two miles southwest of the Cloverbar lagoons (Figure 1).

The volume of wastewater presently treated in the lagoons can best be estimated by the daily water consumption of the various contributors. Combined, Beverly and Oliver use about 1.7 million imperial gallons/day; Sherwood Park, likewise consumes about 1.7 million imperial gallons/day; and the packing plants use near 3.5 million imperial gallons/day during the work week. Thus, during dry weather, the lagoons receive about 6.9 million gallons/day through the workweek and near 3.4 million gallons/day on weekends. This gives a daily average of 5.9 million gallons. Between June 6 and October 2, 1974, an average of 0.8 million imperial gallons/day of sludge supernatant were pumped to

the lagoons between 1600 and 0800 hours, six days a week. This resulted in an average daily flow of 6.6 million gallons. These estimations do not include storm sewer run-off.

The flow patterns at the lagoons during the summer of 1974 are shown in Figure 2. Lime is added to the packinghouse wastes in the city to control odors. Beverly wastes are then added and it takes about five hours for this material to flow to the lagoons. Sherwood Park wastes are added along with more lime at the treatment site. At present, lime is added at a rate of nearly 10,000 pounds/day (170 ppm CaO). The mixed raw sewage then flows through cells 2 and 3 in series. At an average flow rate of 5.9 million gallons/day, the detention time is about 1.3 days in each cell.

The packing plant wastes account for nearly one half of the hydraulic load and the majority of the organic load and contain large quantities of grease and suspended solids. The temperature of the packing plant effluents are about 32°C but after the addition of the cooler Beverly and Oliver wastes the temperature of this sewage when it reaches the lagoons is near 25°C. The Sherwood Park wastes, at near 14°C, are then added and the temperature of the mixed raw influent added to the lagoons is about 22°C. The temperature of the influent material does not vary greatly throughout the year. The effluent from cell 3 however, drops from near

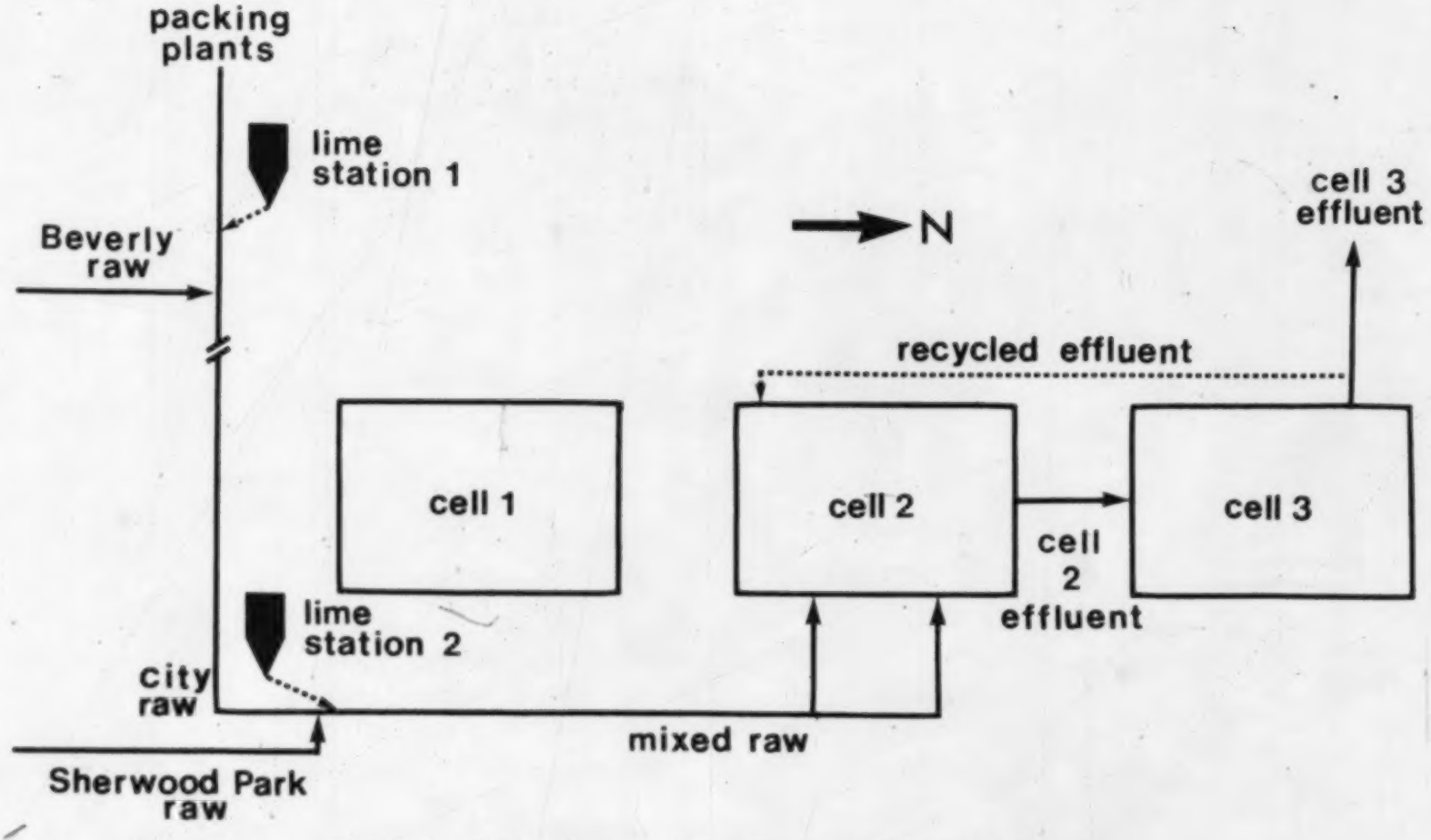


Figure 2. Flow patterns of the Cloverbar industrial lagoons during the summer of 1974.

23°C during the summer, to about 13°C during the winter. The heat supplied by the packing plants effluents is essential to maintain the temperature of lagoons so that the biological processes remain active during the winter months.

Cell 1 has been covered by a scum blanket ever since its start-up. After 15 months of operation, the blanket was reported to be several feet thick in places. Such a blanket decreases the liquid volume of the lagoon, and therefore, decreases its detention time and treatment efficiency. In 1966 and 1968, attempts were made to remove the scum by burning the grease. Self-sustained burning was evident for more than 12 months after the first ignition and for about six months after the second. At present, the grease cover is about two feet thick. Cell 2 has a thin grease cover which is benefical because it serves as an insulating blanket, preventing heat loss during the winter, and it helps to maintain anaerobiosis by reducing aeration due to surface breezes. Cell 3 is scum free because the effluent transfer pipe from cell 2 is located well below its surface and thus very little grease is passed to lagoon 3. Figures 3, 4, and 5 show the surfaces of these lagoons.

The three major operational concerns at the Cloverbar lagoons are:

1. the reduction of the scum blanket on cell 1.

2. the improvement in the overall treatment process to

Figure 3. Surface of cell 1 (looking southeast) October, 1974. The scum blanket was approximately 2 feet thick.

Figure 4. Surface of cell 2 (looking east) October, 1974. The entire surface was covered by a thin grease blanket.

Figure 5. Surface of cell 3 (looking northeast) October, 1974. The bubbles on the surface are a sign of active fermentation. "Scum" in the corner is due to an algal bloom (see page 77).

result in lower strength effluents.

3. the decrease in the production and evolution of odors, at the lagoon site, and in the sewer line en route to the complex.

All of these problems are related to bacterial activities. The first two goals can be achieved by the establishment and maintance of conditions for the balanced growth of non-methanogenic and methanogenic bacteria within the system. The third one involves the suppression of the numbers and activity of the sulfate reducers which under anaerobic conditions, oxidize organic matter and reduce sulfate to sulfide.

Figure 6 illustrates the operational and environmental parameters which are related to the microbial activity in the system and therefore must be considered in the solution of the outlined problems. Some of these parameters can be controlled, others cannot. Loading, for example, cannot be controlled as weekday flow rates are much higher than weekend rates and afternoon and evening flow is greater than morning flow. Loading also affects the temperature and mixing of the lagoon contents. For example, high weekday flows carry the warm packinghouse wastes, whereas, the weekend flow brings only the cooler domestic wastes to the lagoons.

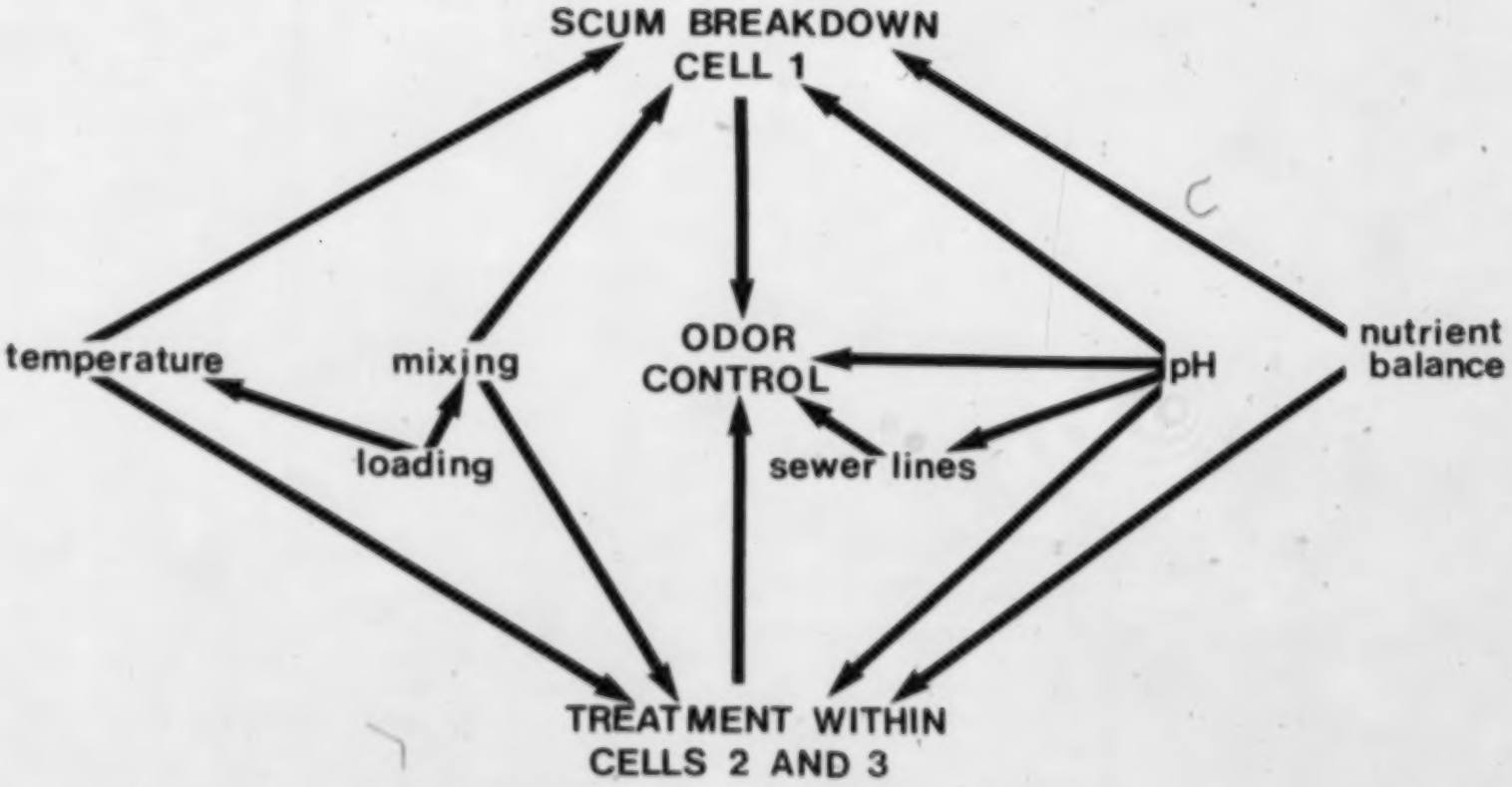


Figure 6. Parameters of major concern during this project (upper case) and influencing factors (lower case).

Mixing enables the microorganisms to come in direct contact with their substrates and therefore accelerates the biological purfication process. However, in a lagoon, much of the BOD reduction results from stratifaction, that is, the settling of solids and the floating of grease. Consequently, a mixing rate must be established which balances both the physical and microbial processes involved in the stablization of wastes.

The control of pH in the lagoons and in the sewer lines is important with regard to both waste purification and odor control. Lime is added to maintain a high pH in the sewer lines which reduces the evolution of hydrogen sulfide, therefore controlling odors. In the lagoons however, the pH must be maintained near neutrality to ensure an active methane fermentation.

As with any biological system, nutrients must be present in correct proportions. To grow efficiently, bacteria require from 10 to 20 parts carbon to 1 part nitrogen. Since the Cloverbar lagoons receive large quantities of carbonaceous material in the form of grease, it is likely that the bacteria, which degrade these, are in a nitrogen deficient environment. One of the aims of this study was to determine whether or not there was an imbalance in the amounts of carbon and nitrogen within the system and if such an imbalance was present, to attempt to adjust their proportions to near 10:1.

The numbers of total aerobes, and sulfate reducers present throughout the system were also determined. Of special interest, with regard to odor control, was the number of sulfate reducers in the sewer line leading to the lagoons and the effects of lime on their viability.

The quantities and proportions of various long chain fatty acids, in all stages of the lagoon system, were determined in order to gain some insight into the chemical nature of the accumulated grease blanket on lagoon 1 and to reveal the fate of these acids in cells 2 and 3.

It was hoped that the data gathered during this study, would lead to the formulation of operational guidelines that would maximize grease breakdown and BOD reduction as well as minimize odors at the Cloverbar industrial lagoons.

LITERATURE REVIEW

I. Anaerobic Digestion

Anaerobic digestion of sewage is a microbiological process in which organic matter is converted to methane and carbon dioxide in the absence of oxygen. This process is widely used for the purification of wastes containing high concentrations of organic material, for example, domestic sewage sludge and industrial wastes from distilleries, meat packing plants, canneries, etc. (Cillie et al., 1969).

McCarty (1964) and Loehr (1967) list several advantages of the anaerobic process over the aerobic treatment. Most notable of these are:

1. It can handle higher loading rates than the aerobic process.
2. There is no oxygen requirement.
3. The production of biological sludge is low.
4. Methane, a useful by-product, is formed.

Since oxygen is not required, an anaerobic process is much less costly than an aerobic system because the installation and continuous operation of aeration equipment is avoided. Also, very little biological sludge or cell mass

is produced because the anaerobic fermentation of organic matter is highly inefficient with regard to recovery of energy available in substrates. Anaerobic microorganisms usually convert between 10 and 20 percent of the substrate carbon to cells, compared to aerobic cultures which convert as much as 50 percent to cellular material (Kirsch and Sykes, 1971). The production of methane, a gaseous fuel which readily separates from the fermentation mixture, is an added bonus in these times of the "Energy Crisis". At present, the methane produced in sewage plants is usually only used to meet the energy needs of the operation. However, many authors, such as Hungate (1974), Gaddy et al. (1974), Stafford (1974), and Savery and Cruzan (1972) are speculating on the feasibility of producing methane as a fuel source from various wastes.

On the other hand, Kirsch and Sykes (1971) and Dague (1972) have pointed out the following disadvantages of anaerobic digestion:

1. Reasonable digestion rate requires a temperature of near 37°C (98°F).

2. Such systems are unable to adjust quickly to changes in nutrient concentration, nutrient composition, temperature or pH.

3. Aromatic compounds cannot be degraded.

4. Anaerobic digestion gives rise to several malodorous compcunds.

Anaerobic digestion is a two-stage process consisting of acid-formation (liquifaction) and gas-formation (gasification). In the first stage, a group of facultative and strict anaerobic bacteria convert the complex organic wastes, such as, protein, carbohydrates, and lipids (grease), to carbon dioxide and volatile fatty acids — mainly acetic acid. In the second stage, these compounds are converted mainly to methane by a unique group of strict anaerobes known as the methane bacteria.

Toerien and Hattingh (1969) state that the terms "acid-formation" and "gas-formation" are misnomers since not only acids are produced as metabolic end-products during the first stage, and not all the gas formed during anaerobic digestion is a result of the second stage. They prefer to call the former, the non-methanogenic phase, and the latter, the methanogenic phase.

Hobson (1973) points out the similarities and differences between an anaerobic digester and the rumen. Both systems must degrade cellulose, starch, proteins, and lipids. The anaerobic digester, however, must also degrade man-made wastes, such as, detergents, dyes, and many industrial compounds. The important difference between the two systems, is that the object of the rumen fermentation is

to maximize the production of volatile fatty acids (acetate, propionate, and butyrate) which are the main carbon nutrient sources for the animal, while minimizing the production of methane, which is an energy loss for the animal. The object of the anaerobic digester, on the other hand, is to maintain maximum production of methane, thereby purifying the wastewaters it receives.

The advances in knowledge of the flora and the biochemical activities in the anaerobic sewage digester has parallelled such advances in rumen microbiology. Because of the similiarities between these two systems, it may be speculated that activities of certain microorganisms and biochemical processes which have, as of yet, only been elucidated in the rumen, may well apply to the anaerobic digester.

Most studies describing the microbiology and/or biochemistry of anaerobic digestion, are based on work with laboratory scale digesters which are maintained at a steady state. Very few reports on full scale digesters are found in the literature.

A. The Non-methanogenic Phase

The non-methanogenic phase includes the biochemical activities of all microbes which do not directly produce methane. This process gives rise to volatile fatty acids and

other low molecular weight, organic compounds by the degradation of the large complex organic molecules present in the raw wastewater. This conversion, however, produces little stablization of the wastes because it is merely a chemical rearrangement of the organic molecules (Pfeffer, 1970).

1. Non-methanogenic bacteria

McKinney (1962) stated that "the acid formers are made up predominantly of facultative bacteria, with a few strict anaerobes." However, Toerien et al. (1967) and Mah and Sussman (1968) used refined anaerobic techniques to show that the strict anaerobes are the more numerous group. Toerien and coworkers studied the flora of seven laboratory-scale digesters which were fed various sterile synthetic substrates to give detention times of 30 days. Over a period of 48 days, strict anaerobes were enumerated using a roll tube technique and facultatives were counted using a standard plate count method. A total of 85 trials gave ratios of obligates to facultatives of between 9:1 and 3575:1. However, the number of obligates, which ranged from 0.1×10^8 to 150.4×10^8/ml, was usually 100 to 200 times that of the facultatives. Methane was not detected in any of the culture tubes, indicating that only non-methanogenic bacteria were counted.

Mah and Sussman (1968) carried out similar studies with

a laboratory digester. The substrate used was domestic raw sewage which was homogenized and frozen in small quantities. Thawed samples were batch fed to the digester at a rate giving a detention time of 20 days. The anaerobic viable counts were between 10^7 and 10^8/ml and were always 10 to 100 times greater than the aerobic counts. However, no attempt was made to discriminate between methanogenic and non-methanogenic colonies.

Domestic sewage may also contain predominantly anaerobes since Mata et al. (1969) showed that in human feces, anaerobes are in the majority over aerobes by 1 to 3 orders of magnitude.

Toerien and Hattingh (1969) summarized the literature since 1900 on bacterial groups found in anaerobic digesters. Toerien (1970) characterized 92 isolates from laboratory digesters, on the basis of their morphology and physiology. The majority were Gram negative rods. Only a few isolates liquified gelatin, hydrolysed casein, or produced indole or acetyl methylcarbinol. Many produced urease and hydrolysed starch. Since identification of the isolates to the generic level was difficult, or even impossible, Toerien concluded that other classification methods should be investigated to determine their value for population descriptions in ecological studies.

One group of anaerobes that should receive greater

attention is the sulfate reducers. These bacteria use sulfate as their terminal electron acceptor, thus reducing it to sulfide. The activities of these organisms produce both desirable and undesirable effects. One benefical aspect, as a result of sulfide production, is the decrease of the redox potential of their environment. Since the second stage of digestion is carried out by the methane bacteria, which are very strict anaerobes, the Eh of the environment must be kept extremely low in order to establish and maintain an active population of these organisms. Therefore, the presence of a strong reducing agent, such as hydrogen sulfide, ($E^{o'}$=−0.243 V; Loach, 1968) is benefical. Martens and Berner (1974) noted that methane in interstitial waters, did not reach appreciable levels until most of the sulfate was removed from the sea water by sulfate reducers. They did not, however, speculate that the increased methane production was due to a more highly reduced environment created by the presence of sulfide.

Sulfide also plays an important role in the removal of heavy metal ions from wastewater. Metals, such as mercury, lead, cadmium, etc., form insoluble sulfides and settle into the sludge. This process protects the anaerobic digester from the toxic effects of the cations and also prevents their escape into the receiving waters. (However, it also limits the agricultural use of the digested sludge.)

The negative aspects of sulfate-reducing activities are

the creation of odor and corrosion problems and the competition with the methane bacteria for molecular hydrogen a key compound in methane formation. Dague (1972) states that hydrogen sulfide is the major component of odors associated with sewage treatment. Some of this odorous gas results from protein fermentation, but most is produced by the activity of sulfate reducers. The role of these bacteria in the corrosion of iron is well documented and is reviewed by Postgate (1960) and by Thistlethwayte (1972).

Postgate (1965) points out that hydrogenase activity is widespread among the sulfate reducers. Most species of methane bacteria obtain energy through the oxidation of hydrogen with the reduction of of carbon dioxide (Bryant, 1974). Therefore, in an anaerobic digester, which has active populations of these two physiological groups, they would, in all probability, be competing for molecular hydrogen. Any such interaction, which decreases the ability of the system to produce methane, is of course, detrimental to the waste purification process.

Using the roll tube technique, Toerien et al. (1968) enumerated sulfate reducers in three laboratory digesters, two of which received domestic sewage sludge and the third received the same sludge plus mine water containing a high amount of sulfate. The numbers of sulfate reducers in the first two were near $5x10^4$/ml while the digester fed sulfate-rich water contained between $6.6x10^5$ and $9.5x10^7$/ml.

These results emphasize that neither the numbers nor the types of bacteria in different digesters can be expected to be the same, since differences in operating conditions, such as temperature, detention time, substrates and so on, will lead to unique populations being established. For example, Mah et al. (unpublished data) showed that the addition of increased concentrations of substrates to an active digester resulted in a population of bacteria which bore no resemblance to the original one.

2. Protoza and fungi

Both protozoa and fungi have been isolated from anaerobic digesters. Lackey (1949) differentiated 18 species of protozoa in an Imhoff Tank, but none were present in large numbers. The role that they play, if any, is as yet, totally unknown (Manwell, 1968) and it is assumed that they are of soil origin. It is also possible that the protozoa feed on the bacteria within the digester, since studies of some rumen protozoa by Gutierrez and Davis (1964) have shown that certain species will not grow unless viable bacteria are included in their medium.

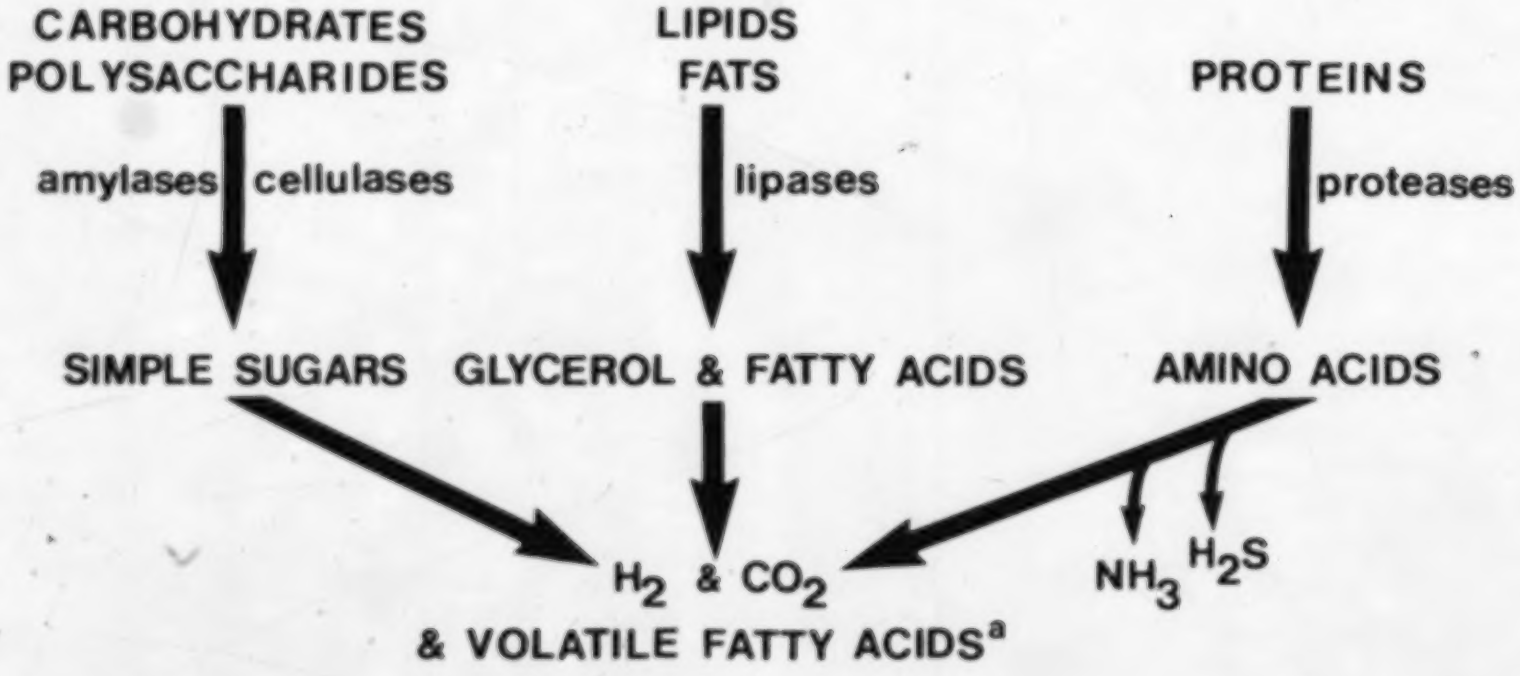
Since the majority of fungi are strict aerobes, McKinney (1962) felt that the fungi isolated from digesters arose from spores which were unaffected by the digestion process. Cooke (1965) reported a study in which large

numbers of filamentous fungi and yeasts were added to a laboratory digester with each batch of influent. Over the study period, there was a gradual decline in the number of species recovered. The fermentative yeasts were the first to disappear while the filamentous species persisted. However, about 15, of the original 66 species, were found consistently throughout the experiment. Pure culture studies have shown that spore production does not occur in the absence of oxygen, therefore, it appears that the filamentous fungi can grow and multiply in an anaerobic digester. The role they play in the digestion process, if any, has not been determined.

3. Biochemistry of the non-methanogenic phase

The majority of the biochemical activities in the non-methanogenic phase are carried out by bacteria. In general, the three main classes of compounds found in sewage are carbohydrates, proteins, and lipids. The amounts and proportions of these vary greatly and are influenced by industrial wastes. For example, brewery wastes contain high concentrations of polysaccharides, whereas abattoir wastes are high in lipids and protein. A general scheme of the breakdown of these materials is given in Figure 7.

Hydrolytic enzymes, such as cellulases, amylases, lipases, and proteases, or bacteria with these respective activities, have been detected in many anaerobic digesters;



[a] Mainly acetate according to Jeris and McCarthy (1965).

Figure 7. Main biochemical activities of the non-methanogenic phase of anaerobic digestion.

Hobson and Shaw (1974), Seibert and Toerien (1969), Kotze et al. (1968), and Toerien et al. (1967). The role of these enzymes is to degrade large polymers into smaller subunits which may be transported into the bacterial cell and metabolized.

The catabolism of carbohydrates in laboratory-scale digesters has been well studied. Kotze et al. (1968) demonstrated the presence of the enzymes fructose-6-phosphate kinase and isocitrate lyase, in the sonicated contents of three digesters receiving wine wastes, glucose-starch wastes, and yeast wastes respectively. The first enzyme indicates the presence of bacteria using the glycoltic pathway and the second is indicative of bacteria utilizing the glyoxylic acid cycle. Only a digester being fed synthetic substrate containing large quantities of carbohydrate material, showed glucose-6-phosphate dehydrogenase activity indicating the presence of bacteria using the hexose monophosphate shunt.

The amino acids which result from the hydrolysis of protein may be used by the bacteria either to synthesize new proteins, or to produce energy for biosynthesis through aerobic or anaerobic oxidation. Since the cell yield is very low, under anaerobic conditions, probably the majority of the amino acids are used for energy production, leaving short chain fatty acids, carbon dioxide, ammonia, and small amounts of hydrogen sulfide as their endproducts. Amino acid

fermentation is reviewed by Decker et al. (1970) and Barker (1961).

Hunter and Heukelekian (1965) and Viswanathan et al. (1962) have shown that triacylglycerols make up a very large proportion of the grease in sewage. Anaerobic digestion of these involves three mechanisms; hydrolysis, biohydrogenation (if an acyl group is unsaturated) and beta-oxidation. Extracellular lipases hydrolyze the triacylglycerols to free fatty acids and glycerol. This conversion is very rapid in an anaerobic digester (Heukelekian and Muller, 1958). Hawke and Silcock (1969) have shown that hydrogenation occurs only after the unsaturated acids are freed from the glycerol moiety. By this mechanism, these acids are converted to their corresponding saturated acids, for example, cis-9-octadecenoic acid is hydrogenated to octadecanoic acid. Wright (1959 and 1960) has shown that in the rumen, bacteria and protozoa are capable of biohydrogenation, therefore both groups are probably active in the anaerobic digester.

Jeris and McCarty (1965), using ^{14}C labelled octanoic and hexadecanoic acids, showed that fatty acids were anaerobically degraded by beta-oxidation to yield acetate. Chynoweth and Mah (1971) have also demonstrated the importance of long chain fatty acids in the formation of acetate. Their laboratory digester, fed domestic sewage sludge, was exposed briefly to various substrates and the

rate of acetate production was measured. They found that hexadecanoic acid was dissimilated to acetate 11 times faster than protein hydrolysate, and more than 80 times faster than glucose or glycerol.

B. The methanogenic phase

The final microbial step in the anaerobic waste purification process is the conversion of the metabolic endproducts of the non-methanogenic bacteria, to methane. At present, the metabolism of the methane bacteria is slowly being elucidated. Their extreme sensitivity to oxygen and their very slow growth rate has made it difficult to isolate pure cultures for biochemical studies.

Bacteria reported to have the capability of producing methane, from various substrates are listed in Table 2. Bryant (1974) feels that the only energy sources utilized by these microbes are hydrogen-carbon dioxide, formate, acetate and carbon monoxide. Those species which are said to use butyrate as a methane-yielding substrate, may not be pure cultures as was the case of the widely studied Methanobacillus omelianskii which was reported to ferment ethanol. Bryant et al. (1967) showed that this "pure culture" was really a mixture of two species. One, the S organism, converts ethanol to acetate and molecular hydrogen. The second, known as Methanobacterium strain

Table 2. Methane - producing bacteria[a]
Family Methanobacteriaceae

Species	Energy Sources
Methanobacterium soehngenii	acetate, butyrate
Methanobacterium formicicum[b]	formate, CO_2 and H_2
Methanobacterium thermoautotrophicum[b]	CO_2 and H_2
Methanobacterium ruminantium[b]	formate, CO_2 and H_2
Methanobacterium mobile[b]	formate, CO_2 and H_2
Methanosarcina methanica	acetate, butyrate (?)
Methanosarcina barkeri[b]	methanol, acetate, CO, CO_2 and H_2
Methanococcus mazei	acetate, butyrate
Methanococcus vannielii	formate, CO_2 and H_2

[a] adapted from Bryant (1974)

[b] reference strain available

M.O.H., reduces carbon dioxide to methane using the hydrogen produced by the S organism.

The stoichiometry of the reductions of carbon dioxide, methanol and acetic acid, to methane, are shown below.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O$$

$$^{14}CH_3COOH \rightarrow {}^{14}CH_4 + CO_2$$

Wolfe (1971) points out that formic acid is also a good substrate for methane formation by most species of Methanobacterium. Present evidence suggests that it is first converted to carbon dioxide and hydrogen and then the carbon dioxide is reduced to methane. However, very little is known about the activation and reduction of carbon dioxide. According to Wolfe (1971), no $^{14}C_1$ intermediates (from $^{14}CO_2$) have been detected prior to the methyl level of reduction which suggests that the activated C_1 unit is firmly bound during reduction.

The importance of formate as an energy source in pure cultures is obvious from Table 2. However, early workers seemed to have overlooked this energy source while concentrating their efforts on the role of acetate which was known to be the endproduct of carbohydrate, lipid, and protein degradation.

McCarty et al. (1963) studied gas production and acid accumulation in the sludge of four digesters. Each was fed a different volatile acid; one formate, one acetate, one propionate and one butyrate. When formate was added to a digester conditioned to one of the other acids, it was utilized very quickly and was never detected in any of the sludges. It was concluded that (a) formate is an important intermediate in the methane fermentation of all substrates and is readily fermented under most conditions or (b) sludges adapted to all other substrates are simultaneously adapted to formate. However, no further studies were preformed to determine the role of formate as an energy source.

Bryant et al. (1971) studied the nutrient requirements of three strains of methane bacteria, two of which were strains of Methanobacterium ruminantium that used formate as their energy-yielding substrate. Both of these, however, needed acetate as a source of cell carbon. Thus, in retrospect, methane formation, in the previously mentioned digesters fed acetate, propionate or butyrate, may have been limited by the scarcity of formate or other suitable energy-yielding substrate.

Pretorius (1972) studied the growth behavior of methanogenic bacteria in completely mixed continuous cultures, when acetate, formate, and a mixture of acetate

and formate were used as sole carbon and energy sources. Three digesters were charged with actively digesting sewage sludge and fed different substrates. The first was fed basal medium plus acetate, the second, basal plus formate, and the third, basal plus formate and acetate. The first digester failed within 60 days, whereas, the others continued to operate under steady state conditions with no detectable concentrations of volatile fatty acids. Thus, formate appears to play a very significant role in methanogenesis.

Smith (1966) enumerated methane bacteria, in three different domestic sewage sludges, according to their energy substrates. In all three, he found 10^7 organisms/ml that would utilize hydrogen. In one he found 10^6 acetate utilizers/ml, while in the other two, he found only 10^5 acetate utilizers/ml. Of these results, Kirsch and Sykes (1971) wrote,

> "It is interesting and at the same time baffling that acetate-fermenting, methanogenic bacteria were found in somewhat lower numbers than hydrogen oxidizing bacteria despite the belief that approximately 70 per cent of the methane produced in a digester can be traced to acetate fermentation [Jeris and McCarty, 1965]. One would want to predict that acetate-fermenting methane bacteria would be predominant forms in the digester environment."

Thus, it appears that the role of acetate as an energy source must be reevaluated in both pure cultures and mixed populations. These studies may also show that the slow

growth rate of the methane bacteria in a medium containing sufficient sources of cell carbon, (i.e. acetate) may be due to the lack of an adequate supply of energy-yielding substrate such as formate or hydrogen-carbon dioxide. If this is true then an anaerobic digester, containing high concentrations of acetate, may actually be lacking a suitable energy source for the methane population.

II. Factors Affecting Anaerobic Digestion

In their review, Kirsch and Sykes (1971) stated that the digestion of wastes preceeds with a minimum of adjustment or control, given a temperature of 29-38°C (85-100°F), exclusion of air, sufficient nitrogen, phosphorous, and trace minerals, a reasonable amount of biodegradable carbon, a pH between 6.6 and 7.5, and the absence of biological inhibitors.

The overall performance of the digester depends on the activity of the methane bacteria and the delicate balance between the non-methanogenic and methanogenic phases. If environmental factors are altered in such a way as to upset this balance, the fermentation becomes uncoupled because the methane bacteria have very stringent physical and chemical growth requirement and are, therefore, very sensitive to environmental changes. Under such conditions, volatile fatty acids accumulate, dropping the pH to a level where the

methane bacteria become completely inactive. A digester in this situation is said to be "stuck". Therefore, the conditions for operation of a digester, are those which are more suitable for the activity of the methane-producers than for the non-methanogenic flora.

A. Temperature

Buswell (1957) reported that methane production could occur from 0 to 55°C. Oswald (1968), felt that the range which was useful in waste disposal was between 15 and 40°C, with an optimum at 32°C. Bryant (1974) reports good growth of various methane bacteria between 30 and 45°C; one thermophile, however, grows best at 65-70°C. According to Oswald (1968) the non-methanogenic group functions between the limits of 4 and 40°C.

B. pH

The literature reports many different pH ranges for methane fermentation — all, however, are near neutrality. Barker (1956) gives a range of 6.4 to 7.2; Pfeffer (1970) says 6.6 to 7.5; Pohland and Mancy (1969) report 6.8 to 7.2; Kirsh and Sykes (1971) feel that "normal" digestion occurs between 6.8 and 7.5; Oswald (1968) gives 6.8 to 7.2, with 7.0 as optimum. Most species listed by Bryant (1974) would appear to fall into these ranges, but one species,

Methanococcus vannielli, grows well between 7.4 and 9.2 with an optimum about 8.0.

In an attempt to determine the tolerance and response of the methane fermentation, Clark and Speece (1970) carried out programmed pH changes on an established population of acetate utilizing methane bacteria. By altering the pH of the influent, they varied the pH of an anaerobic filter, from its normal level (near pH 8), to values ranging from 3.8 to 9.4. Then the selected pH was maintained for various lengths of time (ranging from 12 hours to 21 days), before the filter was returned to its original pH. The activity of the methane producers (and therefore, the efficiency of the purification process) was monitored by measuring the rate of methane production. Clark and Speece concluded that:

1. Steady state acetate fermentation yielding methane at pH levels as low as 4 was possible, although at a rate less than would be attained with the same reactor and bacterial population in the optimum pH range.

2. Acetate fermentation in an anaerobic filter was not inhibited between pH 6 and 8.

3. The recovery of acetate fermentation after a pH drop as low as 5.0 was complete and prompt if the duration was less than 12 hours but it required more time than would be expected based on simple

bacterial regeneration. Also, the recovery is dependent on the pH level to which the digester has dropped (but not the duration of the drop) once the duration exceeds three days.

4. A pH of 9 essentially stopped acetate fermentation, but recovery was complete and prompt after optimum pH levels were restored.

However, they cautioned that their digester response may be atypical because the non-optimum (i.e. non-neutral) pH levels of their preliminary studies may have completely eliminated some pH sensitive acetate fermenting species, leaving only a selected mixed culture which was more pH tolerant than generally found in sludge digestion.

C. Inhibitors

The most obvious inhibitor of anaerobic digestion is oxygen. Smith and Hungate (1958) found that the methane bacteria cannot initiate growth at redox potentials above -0.33 V. Later Hungate (1969) calculated that at this low potential, the concentration of oxygen in water at 30°C in equilibrium with air at one atmosphere pressure is 1.48×10^{-56} molecules/l (assuming that oxygen was the sole factor influencing the Eh).

Other compounds including halogenated methane

analogues, unsaturated fatty acids, and sulfite have been shown to inhibit methane production. Work by Prins et al. (1972) has been directed toward the reduction of methane production in the rumen. Their in vitro studies on the inhibition of M. ruminatium and Methanobacterium M.O.H. showed that methane production was decreased 50% by carbon tetrachloride concentrations of just over $3x10^{-5}$ M, or by cis,cis-9,12-octadecadienoic acid concentrations of $3.2x10^{-3}$ M or sulfite concentrations of $1.2x10^{-3}$ M.

Although the heavy metals are very toxic to both the methanogenic and non-methanogenic bacteria, (Lawrence and McCarty, 1965) they very seldom create a problem since they are usually precipitated as their sulfides. Volatile acids and light metal cations have also been shown to be inhibitory. Much of the work done on cation toxicity has been reviewed by Kirsch and Sykes (1971).

Kugelman and Chin (1971) discuss the roles of synergism and antagonism in toxicity studies. They also evaluate methods used in previous toxicity work, and conclude that these have often given useless and misleading results. They suggest a method of chemostat culturing in a defined medium and the use of the Monod model of growth kinetics (Monod, 1949) to predict the substrate removal efficiency as a function of the biological solids retention time and the concentration of the inhibitor being studied. This appears to be a more reliable a method for studying cation

toxicities since it accounts for their synergistic and antagonistic effects.

D. Nutrients

Kirsch and Sykes (1971) point out that active digestion requires a reasonable amount of biodegradable carbon, sufficient nitrogen, phosphorous, and trace minerals. Various workers have determined the amounts of organic nutrients found in wastewaters, and Kirsch and Sykes (1971) include a review of much of this data.

In a waste treatment process, sewage is the nutrient medium for bacteria whose metabolic activities result in the removal of organic matter and thus the purification of wastewater. Therefore, for maximum treatment efficiency, not only must the nutrients be present, but they must be in correct proportions so that no nutrient is growth limiting. One would speculate that the ideal proportions in the environment would be near the proportions found within the bacterial cell. Of special interest in sewage treatment, is the proportions of carbon, nitrogen and phosphorous (with the latter usually being in excess due to widespread use of detergents containing phosphates).

In their textbook, Stanier et al. (1970) discuss the principles of microbial nutrition. They list the six most plentiful elements in E. coli as a percentage of its dry

weight. These are carbon 50%, oxygen 20%, nitrogen 14%, hydrogen 8%, phosphorous 3%, and sulfur 1%. These figures result in a C:N ratio of near 3.5:1 in the bacterial cell. Alexander (1961) gives the C:N ratio of bacterial cellular components as 5:1, and Brock (1966), gives a value of 10:1 for the C:N ratio of microbial protoplasm. However, for chemoorganotropic bacteria (those which obtain their energy from organic compounds) the fraction of carbon material in the medium should be slightly higher than the fraction in the cell. Jobson et al. (1974) feel that bacteria require about 10 parts carbon to 1 part nitrogen to grow efficiently.

Sanders and Bloodgood (1965) studied the effect of carbon to nitrogen ratio on anaerobic digestion using laboratory-scale fermenters, fed pig food supplemented with hexanoic acid, maltose or L-leucine and various amounts of ammonium hydroxide. They found that the maximum influent C:N ratio which resulted in successful digestion was 16:1.

III. Anaerobic Lagoons

Treating wastewater in lagoons has gained favor over the years, especially in areas where land prices are low and the climate is warm. Anaerobic lagoons were the result of wide spread use of oxidation ponds where increased organic discharges and the greater need for economy pushed the loading rates beyond the natural aerobic stabilization capacity of the ponds (Dornbush, 1971). Thus, the early anaerobic lagoons were merely overloaded aerobic ponds. However, later lagoons were designed specifically for anaerobic digestion, thus giving increased treatment efficiency.

Anaerobic lagoons are widely used to treat industrial wastes (Dornbush, 1971). Probably the leading user of this process is the meat packing industry, whose warm, nutrient-rich wastes help provide favorable temperatures and conditions for anaerobic digestion.

Pfeffer (1970) has likened the anaerobic lagoon to the anaerobic contact process. However, the major difference is the lack of control of the environmental parameters associated with the lagoon. In both systems, a great deal of purification results from the stratification of wastes. That is, the physical separation in which the grease floats to the surface and sludge sinks to the bottom. Dornbush (1970) estimates that this solids separation would account for a

lagoon removal efficiency of 25 to 40% on purely a physical basis. Oldham and Nemeth (1973) used four laboratory, anaerobic contact digesters, at temperatures from 10 to 30°C, to study the effects of various detention times on BOD removal. They determined the overall BOD removal and methane produced by these systems. Using the gas data, they calculated the fraction of BOD removal due to direct degradation. The remainder was due to physical separation. Stratification accounted for only 17% of the total BOD removal by a digester operating at 30°C with a detention time of 50 days. At the other extreme, physical separation accounted for 89% of the total BOD removal by a digester at 10°C with a 25 day detention time.

The presence of a scum or grease blanket is often observed on the surface of an anaerobic lagoon. Such a cover can be detrimental to the system if its volume is so great that it reduces the liquid volume of the lagoon. This decreases its detention time and treatment efficiency. However, if only a thin blanket exists, it can be beneficial to the treatment process by decreasing aeration due to surface breezes and in cold climates serving as an insulating layer to reduce heat losses. Bolag and Dornbush (1966) reported that the original design of an anaerobic lagoon system in Minnesota included the installation of an underwater gas-fired heater. However, the grease blanket which formed, insulated the ponds so well that the heater

was never installed. Etzel (1964) reported that an artificial cover of floating plastic foam, three inches thick, was constructed on a lagoon treating corn product wastes in Indiana. As a result, the winter lagoon temperature increased from near 16° to greater than 30°C.

Coerver (1964) noted that lagoons treating packinghouse wastes, in Louisiana, operated nuisance free only after a "crust" had formed, therefore suggesting that a scum blanket helps to reduce odors. However, Meyer (1965) has observed that lagoons treating similiar wastes have operated without nuisance odor, in the absence of such blankets.

METHODS AND MATERIALS

I. Sampling

Figure 8 shows the locations where grab samples of the influents and effluents of the Cloverbar industrial lagoon system were taken. Since the strength of the wastes varies greatly according to the time of day and week, sampling times are always reported. The packing plant wastes and the Sherwood Park wastes were taken from manholes using a plastic pail on a rope. The pail was filled once and rinsed, then a second full pail was drawn and its contents transferred to a sterile 4 l, wide-mouth plastic bottle. The remaining samples were taken from the concrete channels as shown in Figures 9 and 10. These samples were recovered by dipping a sterile 4 l jar into the wastewater. The city raw and mixed raw samples were subsurface samples, taken in such a manner as to avoid sampling only the grease material floating on the surface.

Samples used for bacterial counts were taken in duplicate or triplicate. However, only single samples were taken when monitoring the effects of recycling (to be discussed later).

Samples were obtained from several locations in all three lagoons. The surfaces were charted so that sampling locations could be readily located (Figure 11). Samples of

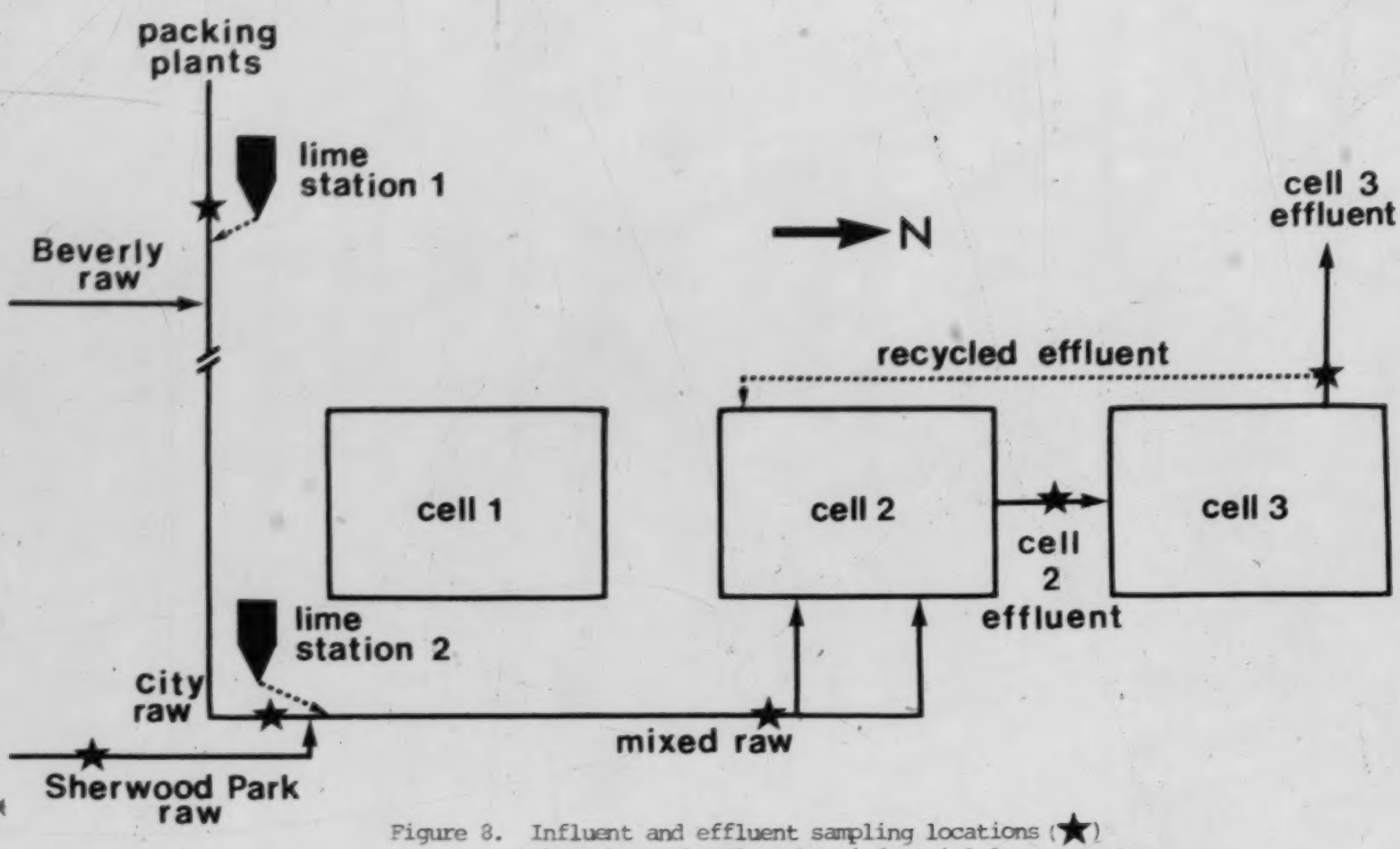


Figure 8. Influent and effluent sampling locations (★) throughout the Cloverbar industrial lagoon system.

Figure 9. Channel carrying mixed raw sewage. The rod on the float is about 3 feet long.

Figure 10. Channel carrying cell 3 effluent. The treated wastes leave the lagoon (bottom right of photo) and travel through the channel (upper right) to the storage ponds and outfall. Channel (lower left) carries effluent for recycling to cell 2.

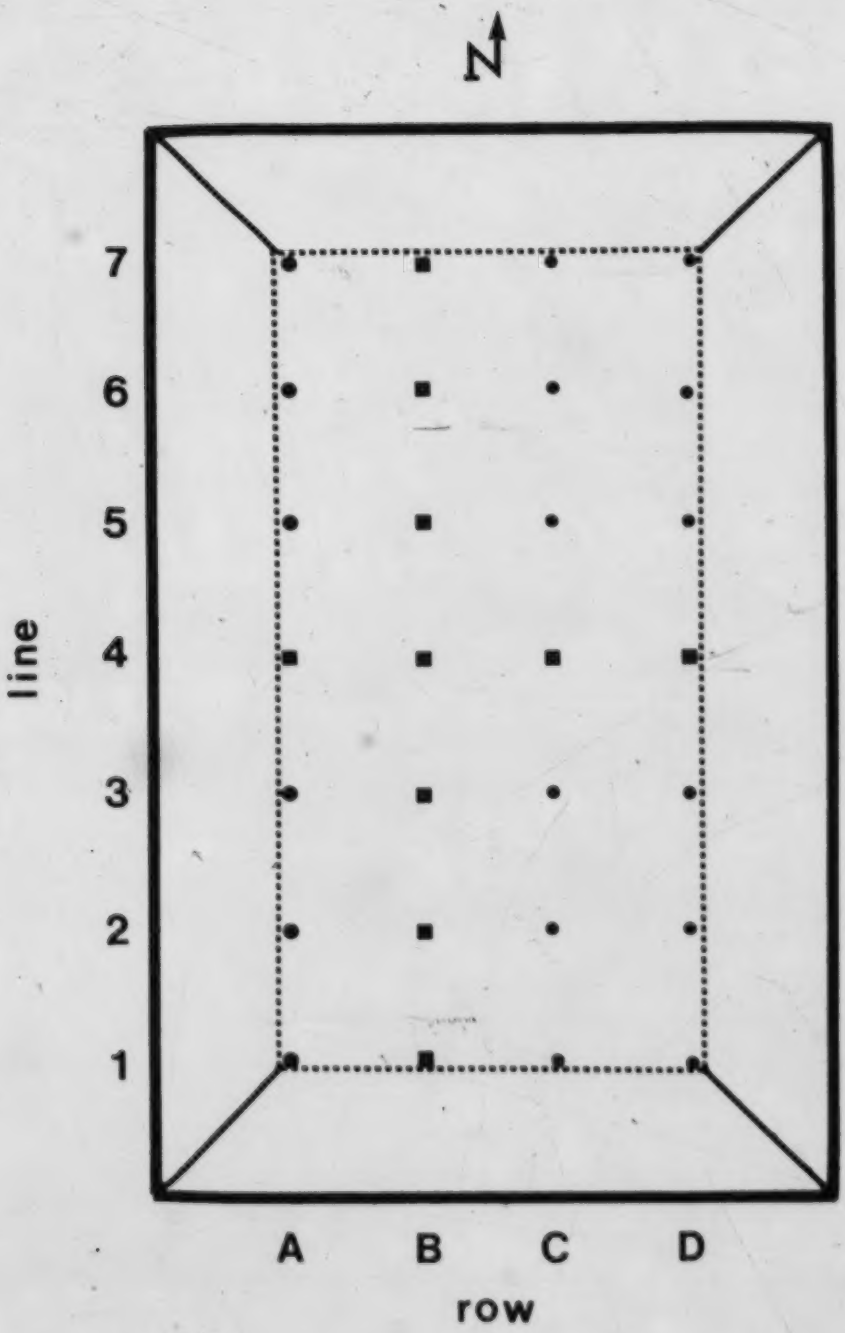


Figure 11. Surface of a lagoon charted for sampling. (■) Indicates sample locations.

three strata; the surface, the 12 foot depth (called middle), and the bottom were taken at each location marked with a square. The boat used for sampling (shown in Figure 12) contained a "well" in its middle through which samples were taken.

A surface sample was taken at each location of cells 1 and 2. These were collected in sterile, plastic, 250 ml, wide-mouth bottles and were pooled giving one, composite sample, from each lagoon, for microbial and chemical analysis. Four hundred ml of surface water from each grid point on cell 3 were gathered in a sterile graduated cylinder and pooled in a sterile 4 l sample bottle.

Subsurface samples were taken using the device shown in Figure 13. The handle was marked off at 2 foot intervals. The "can" was lowered to the desired depth, and the rope was pulled, opening the top and bottom lids. When the rope was released, the lids would seal, enclosing a sample from that depth.

Four hundred ml of middle sample were taken at each location and pooled giving one composite sample from each lagoon. The composite bottom samples were made by combining 250 ml volumes of sludge gathered at each grid point. While still at the lagoon site, the surface and middle composites were homogenized in a sterile container with a Waring Blender and the heavy sludge samples were well mixed with a

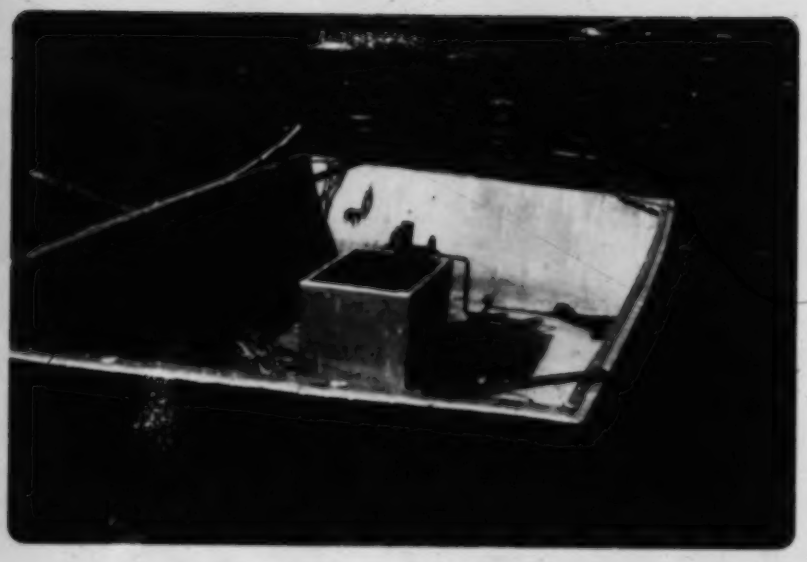


Figure 12. Boat used for lagoon sampling.

Figure 13. Depth sampling device used to obtain middle and bottom samples.

sterile spatula. Samples were then transported in an ice chest back to the laboratory. Material required later, for chemical analyses, was stored at −20°C.

II. Bacterial Counts

Bacterial counts were carried out on influent, effluent and lagoon strata samples. The total aerobes present were determined using the spread plate technique on PCA (Difco) after an incubation period of 4 days at 25°C. The number of sulfate reducers was determined using a five tube most probable number (MPN) technique in tubes (16x150 mm) containing 10 ml modified Butlin's broth (see Appendix) and two iron nails which lowered the redox potential of the medium. The inoculated tubes were incubated at room temperature for 14 days and then scored for the presence of sulfate reducers as indicated by a blackening of the nails.

Due to the high pH of some samples (as a result of lime addition), all dilutions were carried out in the field immediately after the samples were taken. Dilutions used for plate counts, were made in 0.3 mM phosphate buffer, pH 7.2 (American Public Health Association [APHA], 1971). The dilution buffer used for the sulfate reducers was 0.6 mM phosphate buffer containing 0.1% sodium thioglycollate (pH 7.2). Nine ml of this buffer was autoclaved in sealed Hungate tubes (Bellco Glass Inc., Vinland, N. J.) and dilutions were carried out using sterile 1 ml syringes.

The diluted samples were transported (at ambient temperature) to the laboratory, where the growth media were inoculated within six to eight hours after sampling.

III. Physical and Chemical Analyses

The temperature and pH of samples were determined at the sampling site. The pH of the strata composite samples were measured in the field as soon as the mixing was completed. A portable Radiometer pH meter 29, which was standardized with buffer at pH 7.0 or 10.0, (depending upon the pH of the sample) was used throughout this study.

Total solids, volatile solids, suspended solids, and volatile suspended solids were determined as outlined by the APHA (1971), using, in most cases, sample volumes of 50 ml. However, only 20 ml of sludge and scum samples were required for the total and volatile solids determinations. Aliquots of these were measured by means of a disposable, plastic, 20 ml syringe with the tip bored to a larger diameter to minimize plugging by particulate matter present in the samples.

To evaluate the balance of microbial nutrients in the system, the quantities of carbon, nitrogen, and phosphorous were determined. Inorganic and total carbon were measured using a Beckman Model 915 Total Organic Carbon Analyzer. Samples were homogenized for .5 minutes in a Sorvall

Omni-Mixer, and then sonicated for 2 minutes prior to carbon analysis. Ammonia and organic nitrogen concentrations were determined using a macrokjeldahl apparatus. The amount of ammonia, trapped in the boric acid, was determined by titrating with standard sulfuric acid. Total phosphate analyses were carried out using the vanadomolybdic acid method after digestion with sulfuric acid - nitric acid. All three methods are outlined by the APHA (1971).

Biochemical oxygen demand (BOD) was measured by the procedure of APHA (1971). Dissolved oxygen was measured by polargraphic methods using the dropping mercury electrode, (Southern Analytical Dissolved Oxygen Meter).

Grease content was determined using a 4 hour Soxhlet extraction with Freon TF as the solvent. The methods used for wastewaters and for sludges are outlined by the APHA (1971). Scum samples were analyzed for grease in the same manner as the sludge samples.

The presence and concentration of long chain fatty acids were determined by gas liquid chromatography. Methyl esters of the fatty acids were prepared by gently refluxing 20 to 50 mg of grease with 2 ml of 1% (w/v) sulfuric acid in methanol for two hours. After methylation, the contents were diluted with water and the esters were extracted with n-hexane, washed twice with 0.2 N potassium bicarbonate and finally with water. The residual water was removed with

anhydrous sodium sulfate and the hexane was evaporated under a stream of nitrogen, (Given et al., 1974). This residue was dissolved in a suitable volume (usually 1.0 ml) of methylene chloride and injected onto a 6 ft x 4 mm glass column packed with 15% Ethylene Glycol Succinate on AW 100/120 mesh Cromosorb P (Supelco Inc., Bellefonte Pa.).

The gas chromatograph used was a Hewlett-Packard Model 5700A equipped with a flame ionization detector. The injection port, column, and detector temperatures were 200°, 185°, and 250°C respectively. Nitrogen was used as the carrier gas at a flow rate of 60 ml/min.

Ettre and Kabot (1963) showed that the flame ionization detector gives a relative response of very nearly unity for equal weights of methyl esters of long chain fatty acids. Thus, methyltetradecanoate (Applied Science Laboratory Inc., State Colege, Pa.) was used as a quantitative standard and peak areas were determined using a Hewlett-Packard 3370B Integrator.

IV. Chemical Treatments of the Cell 1 Scum Blanket

Attempts were made to hasten the microbiological degradation of the scum on lagoon 1 by chemically treating portions of the blanket. Wooden frames, one meter square, were constructed and dropped on the surface, forming a "plot". A piece of styro-foam hinged to a yardstick, was pushed through the scum in the middle of the plot. The hinge was then opened and the yardstick released. This left the styro-foam floating up against the bottom of the scum and the yardstick in a nearly vertical position, allowing the measurement of the depth of the scum.

Three chemically treated plots and an untreated control were set up in duplicate on two occasions. One of the treatments used, was lime added at a rate of 2000 pounds/acre (2 pounds/plot). Another treatment involved the addition of urea to give a C:N ratio of near 10:1 in the scum. A commercial product know as "Micro Aid" (which is claimed to cure scum problems) was used as a third treatment. Five ml of this product were diluted in water and sprayed over the surface of the plot. Figures 14 and 15 illustrate the plots.

Figure 14. A plot treated with lime. Scum depth measures 7 inches.

Figure 15. Six of the eight plots on the surface of cell 1. Treatments are (from left to right) control, urea, control, urea, lime, lime.

V. Varying the C:N Ratios in Cells 2 and 3 by Recycling Final Effluent

When it was found that the C:N ratios were too high in cell 2 and too low in cell 3, attempts were made to adjust these to values closer to 10:1. Nitrogen could be supplied to the nitrogen-deficient cell 2 by recycling part of the cell 3 effluent (which had a low C:N ratio) through the second lagoon. At the same time, if the volume recycled was great enough, the increased flow rate through cell 2 should decrease the stratification of the suspended material. Thus, increased amounts of carbonaceous matter should flow into cell 3 (the carbon deficient lagoon). By this means, the C:N ratios in both cells could be corrected.

With the average daily influent flow of near 6 million gallons, it was speculated that a recycle volume of 1 million gallons/day would bring about the desired effects. A pump was installed to lift this volume of the cell 3 effluent from the outgoing channel carrying it back to cell 2.

Samples of cell 2 and cell 3 effluents were taken during a four week period before recycling and for an additional four weeks during recycling. Since the quality of the effluents varies during the week, samples were taken on Thursdays (characteristic of weekday flow) and Sundays (as an example of weekend flow). Grid samples were taken from

both lagoons one week before and four weeks after recycling had commenced. The pH and temperature of each sample was recorded and each was analyzed for total solids, volatile solids, suspended solids, volatile suspended solids, grease, BOD, ammonia and organic nitrogen, and inorganic and total carbon.

RESULTS AND DISCUSSION

I. Microbiological Studies

A. Evaluation of Dilution Methods used for Bacteriological Counts

The total aerobic and sulfate reducer counts of the influent samples taken in July, 1973, are presented in Table 3. At that time, lime was being added to the wastewaters at an average rate of 12,200 pounds/day. All samples were transported, undiluted, back to the laboratory where they were diluted and plated within three to four hours after the last sample was obtained. The low viable bacterial count found in the city raw wastes (pH 11.9) was expected since this material had been at a high pH for about five hours (the approximate flow time from the packinghouses to the lagoons). However, the low numbers found in the mixed raw, were quite unexpected. About three volumes of city sewage are mixed with one volume of Sherwood Park wastes and this mixed raw sewage, at a liberal estimation, flows for 10 minutes in the channel before reaching the sampling location. Thus, it seems unlikely that the 1 in 4 dilution of the Sherwood Park wastes containing 7×10^4 organisms/ml, could be decreased to 10^3/ml by such a short contact time at the elevated pH. This drastic reduction in viable organisms implied that transportation of undiluted samples was unsatisfactory since it increased the contact time by at

Table 3. Initial bacterial counts of the influents to the Cloverbar industrial lagoons[a]

Sample location	Time	pH	Aerobic Count millions/ml ± LSD	Sulfate Reducers[b] thousands/ml ± LSD
Packing plants	0900	6.4	41 ± 13	24 ± 18
City raw	1115	11.9	0.00047 ± 0.00005	< 0.1
Sherwood Park raw	1045	7.5	7.0 ± 2.6	17 ± 6.4
Mixed raw	1130	11.9	0.0010 ± 0.0004	< 0.1

[a]Samples taken Thursday, July 19, 1973 and transported back to the laboratory for diluting and plating.

[b]MPN diluted in non-prereduced buffer

least three hours.

To determine if the loss of viability which occurred during transport could be reduced, aerobic counts on a mixed raw sample (pH 11.1) diluted in the field, were compared to counts on the same sample transported undiluted. The former sample had nearly 40 times more viable organisms than did the latter. Dilution of the samples in the field effectively lowered the pH to near neutrality. For example, the pH values of the 10^{-1}, 10^{-2} and 10^{-3} dilutions were 9.8, 7.9 and 7.3 respectively.

The change in numbers of total aerobes in diluted and undiluted samples as a function of time was also determined. To eliminate the adverse effects of high pH, a mixed raw sample was taken when very little lime was being added (pH 7.7). Table 4 summarizes the results of diluting and plating in the field; diluting in the field and plating in the laboratory at two different times (3 and 6 hours); and diluting in the laboratory and plating in the laboratory 3.5 hours after sampling. The first trial (i.e. diluted and plated at the lagoons) has been assigned a relative count of 1.0. Those samples diluted in the field and plated in the laboratory after 3 and 6 hours, have relative counts of 0.64 and 0.55 respectively. Thus the numbers of viable organisms decrease slightly when the diluted sample is transported and stored before plating in the laboratory. The sample which was diluted and plated in the lab, had a relative count of

Table 4. Effects of dilution and transportation time on the numbers of viable bacteria in a raw sewage sample[a]

Diluted at	Plated at	Time between Sampling and Plating (hr)	Aerobic Count[b] millions/ml ± 1SD	Relative Count
lagoons	lagoons	0.1	11 ± 1.3	1.0
lagoons	laboratory	3	7 ± 0.3	0.64
lagoons	laboratory	6	6.1 ± 0.7	0.55
laboratory	laboratory	3.5	18 ± 0.07	1.67

[a] pH 7.7

[b] average of two samples

1.67, suggesting that growth was occurring in transit. Therefore, during transport of a sample with a near neutral pH, the number of viable bacteria changes regardless of whether the dilutions were done in the field on in the laboratory. However, since the transport of an undiluted sample with a high pH, gives a considerable decrease in viable counts, all later samples were diluted in the field and plated in the laboratory as soon as possible after sampling.

The effects of dilution methods on the determination of sulfate reducer MPN's was also evaluated. The buffers and techniques studied are outlined in Table 5. After autoclaving, the pH of buffer containing 0.1% (w/v) sodium thioglycollate and 0.3 mM phosphate was below 7. Thus, 0.6 mM phosphate was used to maintain the pH at 7.2. The effects of buffers, dilution techniques and sample storage time, on the numbers of sulfate reducers found in a sewage sample, with a pH near neutrality, are summarized in Table 6. The methods used do not appear to affect the results significantly. However, since method 2 provides a reasonably reduced environment and eliminates the time for degassing, it was the method used for the latter part of the study. Dilutions were done in the field and inoculations made in the laboratory on the day of sampling.

Table 5. Evaluation of dilution techniques and buffers[a] for determination of sulfate reducer MPN

Method	Sodium thioglycollate % (w/v)	Degassed with N_2 before autoclaving	Container	Aliquots transferred by
1	0.1	Yes	Hungate[c] tubes	syringe
2	0.1	No	Hungate tubes	syringe
3	0.1	No	Milk dilution bottles	pipette
4	0	No	Milk dilution bottles	pipette

[a] all buffers contained 0.6 mM phosphate (pH 7.2)

[b] using a 5 tube method

[c] Bellco Glass Inc., Vinland, N.J.

Table 6. Effects of various dilution buffers, techniques and sample storage times on numbers of sulfate reducers in a sewage effluent[a]

	MPN millions/ml ± LSD		
	Sample Storage Time		
Method[b]	0 hr	5 hr	3 days
1	2.8 ± 0.64	6.7 ± 6.1	5.6 ± 3.2
2	1.6 ± 1.1	2.8 ± 0.64	7.2 ± 5.4
3	1.2 ± 0.64	2.3 ± 1.4	2.8 ± 2.9
4	0.41 ± 0.11	0.79 ± 0	1.3 ± 0

[a] pH 7.4

[b] see Table 5 for description

B. Effects of Lime on Bacterial Counts in the Sewer Lines

Lime is added to the packinghouse wastes in the city and to the mixed raw in the influent channel prior to entry into the lagcons to control odors. Both of these additions help to maintain the pH of the lagoon contents near neutrality.

Chemically, lime controls odors by increasing the pH, thus trapping hydrogen sulfide as an ionic species (HS^- or S^{2-} depending upon the pH). Figure 16 shows the amount of each species present a various pH values. At pH 9, there is virtually no sulfide in the gaseous form.

As shown in Table 3, during the early stages of this study, the weekday pH value of the city raw and mixed raw was near 12. The large decrease in the number of sulfate reducers suggested that liming not only controls odors by chemical means, but also by biological means. That is, the high pH appeared to be toxic to the sulfate-reducing bacteria which are responsible for the production of the hydrogen sulfide gas.

The strength of the wastes reaching the lagoons between July, 1973 (time of sampling for data in Table 3) and May, 1974 (data in the upper half of Table 7), decreased due to process modifications at the packing plants. For example, in July, 1973, the average BOD of the mixed raw was 970 mg/l,

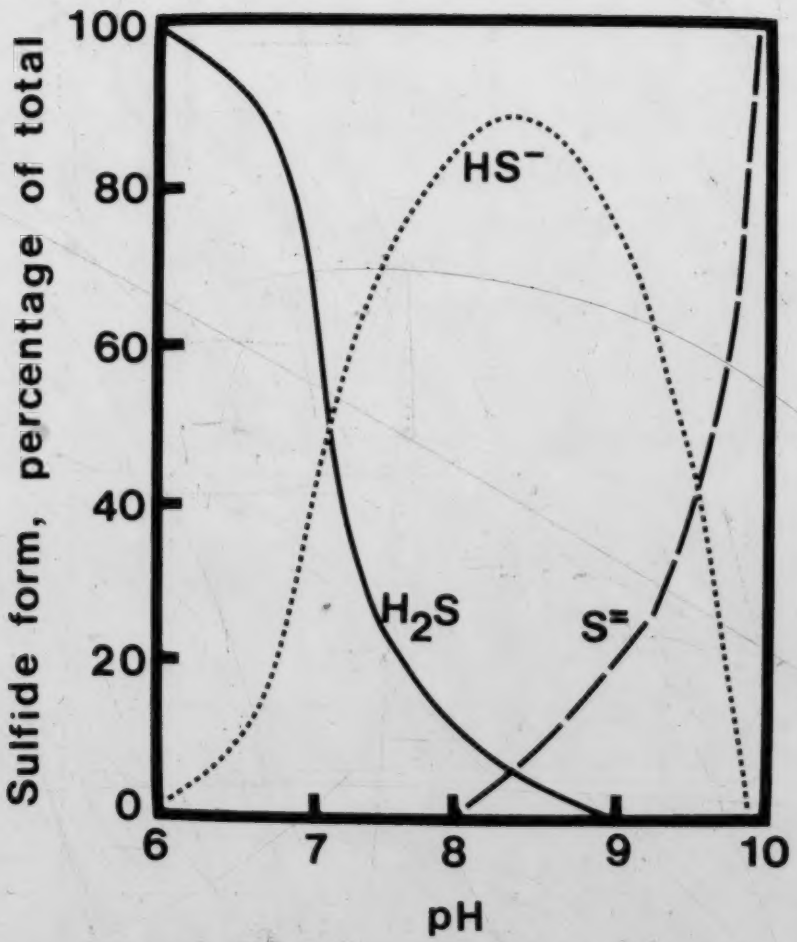


Figure 16. Forms of sulfide in aqueous solution at various pH values. After Sawyer and McCarty (1967).

Table 7. Effects of lime on viable bacterial counts in the sewer line between the packing plant and the Cloverbar lagoons

Sample	Date[a]	Time	pH	millions/ml ± 1 SD	
				Aerobic Count	Sulfate Reducers
Packing plant wastes[b]	May 8	1120	6.8	5.4 ± 0.59	27 ± 23
City raw	May 8	1600	9.3	9.4 ± 1.2	42 ± 20
Packing plant wastes[b]	Oct 17	1030	6.9	20 ± 1.3	0.18 ± 0.06
City raw	Oct 17	1515	11.8	0.22 ± 0.02	0.0095 ± 0.0021

[a] 1974

[b] sample taken from manhole just before lime station

whereas, the corresponding value for May, 1974 was 665 mg/l. The suspended solids likewise decreased from 1026 to 505 mg/l (City of Edmonton Waste Treatment Facilities Monthly Reports). The amount of lime needed to maintain a near neutral pH in the lagoons decreased, as a result of the reduced loading. Hence, the rate of lime addition was decreased accordingly and the pH values observed were near 9.5 (about 2 pH units lower than those observed in July, 1973).

Viable bacterial counts were carried out with samples taken in the city, before liming, and with the city raw samples taken five hours later at the lagoons to determine whether the high rate of lime addition in the city was bacteriocidal to the aerobes and sulfate reducers during the flow time to the system. All dilutions were made in the field as previously described. The results of two different sampling days are summarized in Table 7. In May, 1974, the pH of the city raw was 9.3 (typical of samples taken during the summer of 1974) and no decrease in counts was observed. Approximately 24 hours prior to the second sampling trip, the rate of lime addition was increased to raise the pH of the city raw to 11.8 which was similiar to the values observed during the summer of 1973. This high value decreased the viable counts only slightly (about two orders of magnitude). Those least affected were the sulfate reducers. Thus, it appears that even the very high rate

liming does not extensively kill the organisms responsible for the production of hydrogen sulfide. However, the elevated pH may inhibit their rate of sulfide production by decreasing their overall rate of metabolism.

The low numbers of sulfate reducers found in the city raw and mixed raw samples taken July 19, 1973 (Table 3) are probably due to incorrect sample handling. That is, these samples (pH 11.9) were not promptly diluted nor were they inoculated into the Butlin's broth on the day of sampling.

The results in Table 7 agree with the report by Bunker (1942) that sulfate reducers can survive up to pH 11.7, becoming active when the pH drops. Skinner (1968) reports that these bacteria are active between pH 5.5 and 9.0. Their optimum being near neutrality.

Hence, lime should be added in the city at such a rate as to maintain a pH of 9 throughout the sewer line. This will chemically eliminate hydrogen sulfide odors. Lime addition at any higher rate would neither be benificial nor economical.

C. Numbers of Viable Bacteria in the Lagoon System

The bacterial counts of the influent and effluents of the Cloverbar lagoons are presented in Table 8. The pH values of the three samples are typical of those observed during the later stages of this study. These data indicate

Table 8. Viable bacterial counts of the Cloverbar lagoons influent and effluents

Sample	pH	millions/ml ± 1 SD	
		Aerobic Count	Sulfate Reducers
Mixed raw[a]	9.1	32 ± 0.71	1.4 ± 1.3
Cell 2 effluent[b]	7.2	8.2 ± 2.5	0.36 ± 0.18
Cell 3 effluent[c]	7.3	1.7 ± 0.25	0.18 ± 0.07

[a]Sampled 1300 Monday, August 26, 1974.

[b]Sampled 1300 Wednesday, August 28, 1974.

[c]Sampled 1415 Thursday, August 29, 1974.

that there is only a very slight decrease in the bacterial numbers as the wastes pass through the lagoons. It is interesting to note that the numbers of sulfate reducers essentially remains constant throughout the system.

The numbers of bacteria found in the strata of all three lagoons are given in Table 9. A comparison of the numbers at the surfaces of the three cells, shows that the two lagoons which are covered by grease blankets have substantially larger populations than does the third which has no scum. The large numbers of total aerobes (which also includes facultative anaerobes) and the slightly lower pH of the scum blanket, would indicate an active non-methanogenic population. The bacterial densities in the first two lagoons are lowest in the middle, whereas, the lowest density in the third is at the surface.

The numbers of sulfate reducer are relatively uniform throughout the lagoons. The high counts at the surfaces, especially in cell 1, suggest that the redox potential of the lagoons is low — even at the surface — since the majority of these organisms require an Eh of less than -100 mV to grow (Postgate, 1974).

Table 9. Viable bacterial counts of various strata of the Cloverbar lagoons

Sample		pH	Aerobic Counts millions/ml ± 1 SD	Sulfate Reducers millions/ml (95% Confidence Limits)[a]
Cell 1[b]	Surface	6.2	450 ± 30	70 (23/170)
	Middle	7.8	0.76 ± 0.09	0.49 (0.17/1.3)
	Bottom	7.3	4.3 ± 1.1	1.3 (0.35/3.0)
Cell 2[c]	Surface	6.4	79 ± 15	3.3 (1.1/9.3)
	Middle	7.2	18 ± 2.0	0.49 (0.17/1.3)
	Bottom	7.1	55 ± 20	2.4 (0.68/7.5)
Cell 3[d]	Surface	7.6	0.47 ± 0.04	0.13 (0.035/0.30)
	Middle	7.6	1.1 ± 0.08	0.33 (0.11/0.93)
	Bottom	7.0	7.0 ± 1.1	0.49 (0.17/1.3)

[a] (lower/upper)

[b] Sampled Wednesday, June 5, 1974. No flow through cell 1

[c] Sampled Wednesday, August 28, 1974.

[d] Sampled Thursday, October 3, 1974.

D. Odor and pH Control at the Lagoons

Dague (1972) states that the most common odor in wastewater systems is due to the formation of hydrogen sulfide. During a study of odor and gas production at the Cloverbar lagoons, Coutts (1972) reported hydrogen sulfide concentrations between 945 and 2550 ppm in the gases evolved from cell 3. Values of 1260 and 1380 ppm were obtained in gases from cell 2 during the summer of 1974 (Coutts, personal communication).

The evolution of hydrogen sulfide can be decreased simply by increasing the pH of the lagoons. This however, would be detrimental to the activity of the methane bacteria. For example, the data of Given (1972), (Figure 17) illustrates how sharply the production of gas in cell 3 decreases when its pH was adjusted to non-neutral values. (Unfortunately, the amount of methane present in the gas was not reported.) The rate of grease breakdown, which is also a function of pH, must be kept as high as possible for two reasons. First, the rate must be great enough to ensure that no further scum accumulation occurs on cells 1 and 2; and second, to ensure the presence of substrates for the methane bacteria. Given et al. (1974) reported that past experience at the Cloverbar lagoons has shown that grease accumulated when the operational pH was below 7.5.

A single lagoon cannot be maintained at a pH which

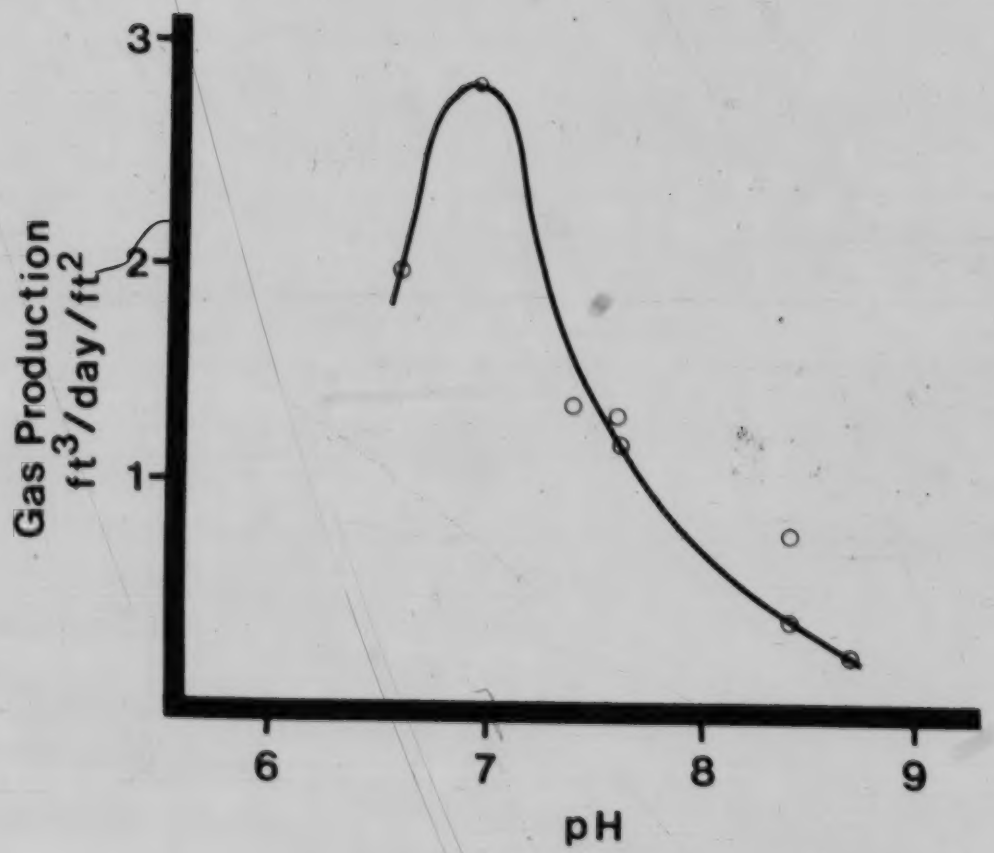


Figure 17. Total gas production from lagoon 3 as a function of pH. After Given (1972). Gas analyses were not reported.

would give favorable rates of both methane production and grease breakdown, while keeping hydrogen sulfide liberation to a minimum. However, by using two lagoons in series, (as is the case with the Cloverbar lagoons) the pH of the first could be maintained at values near 7.5 to increase the rate of grease breakdown and decrease the hydrogen sulfide odors (at the expense of decreased methane production) while the pH of the second lagoon in series is kept between 7.0 and 7.2 to maximize methane formation. By this means, the non-methanogenic bacteria would be the more active group in the first lagoon and the methane bacteria would be active mainly in the second lagoon. The metabolic endproducts from the non-methanogenic phase would be carried into the second lagoon where they would be reduced to methane.

The above system should work well in warm climates or during the summer months. However, the proceedure should be altered during the winter months in Alberta. Since the methane population is more cold sensitive than the non-methanogenic group, it must be protected during the winter. The first lagoon in series is obviously the warmer of the two and therefore, will be more favorable for the methane population. As a further protective step, both lagoons should be kept near pH 7 throughout the winter to ensure the survival and activity of the methane bacteria.

Figure 14 shows that at pH values near 7.2, about 50% of the sulfide present is in its volatile form. Rather than

using pH control to trap hydrogen sulfide (as an ionic species), it may be precipitated by heavy metals. Dague (1972) discussed the addition of various metal ions (ferric, ferrous, zinc, copper, etc.) to sewage for this purpose. Such a process would be quite expensive and there is the danger that some of the cations may pass through the system into receiving waters. A much less costly method of trapping hydrogen sulfide, is as ferrous sulfide, by the addition of scrap iron to the lagoons. Also, iron is relatively non-toxic and if it passed through the lagoon system, it would not grossly affect the quality of the receiving waters.

While studying the production and control of hydrogen sulfide from anaerobic swine manure, Barber (1974) tested the capabilities of various forms of iron to trap the odorous gas. Included were ferrous, ferric, and powdered iron as well as iron rods. The findings showed that all four forms did reduce the amount of sulfides released from the fermenting manure. The ionic species and the powder were most effective, trapping 50% or more. Whereas iron in the form of a rod trapped only very little hydrogen sulfide. Since equal weights of metallic iron were used, either as powder or as a rod, it is obvious that the trapping efficiency increases as the surface area of the metal increases. Therefore, scrap iron added to the lagoons should have considerable surface area. Automobile bodies, stripped

of chrome etc., or metal lathe turnings would be ideal.

Many authors of review articles on anaerobic lagoons refer to Coerver (1964) who implies that a scum blanket helps reduce odors. In a report to the City of Edmonton, Given (1972), suggested that the formation of a 6 to 12 inch scum layer on both cells 2 and 3 may help minimize hydrogen sulfide odors. The data in Table 9 however, indicate that such a scum blanket may add to this problem for two reasons. Firstly, there is a large population of sulfate reducers in the blanket and secondly, the pH of the scum is always lower than that of the rest of the lagoon contents. As an example, the average pH of the surface samples of cells 1 and 2 is 6.3 (from Table 9), and Figure 16 shows that, at this pH, 95% of the sulfide present is in the volatile form.

E. Appearance of Algae during a Period of Reduced Loading

An algal bloom occurred on the surface of cell 3 in early August, 1974 (see Figure 5). This followed a labor dispute at the packing plants which forced their closure for most of June and July. During this period, the lagoons treated only domestic wastes and the reduced load created and environment suitable for a species of Oscillatoria to flourish. These blue-green algae are often found in moderately polluted waters (APHA, 1971), but it is unlikely

that they would occur under normal loading conditions nor that they would survive the cold winter.

II. Attempts to Increase the Rate of Grease Digestion in the Cell 1 Scum Blanket

In order that cell 1 may be returned to service, conditions must be established to hasten a rapid microbiological degradation of the massive grease blanket. After this, conditions must be maintained to prevent the reoccurance of a grease build-up.

Plots, set up on two different occasions, were used as small scale studies to determine whether the addition of lime, urea, or "Micro Aid" would increase the rate of grease digestion. However, no results were obtained from either experiment because the liquid contents of the lagoon were "lost" on both occasions. The exact cause of the liquid loss is still unknown, however, it is thought that there was a malfunction in the sludge recirculating system which caused the contents of the first lagoon to be pumped into the second lagoon.

III. Results of Chemical and Physical Analyses of Wastes from the Cloverbar Lagoon System.

A. Preliminary Analyses

The chemical and physical analyses of typical influents and effluents of the Cloverbar industrial lagoons are presented in Table 10. Those influent samples taken prior to the addition of lime have near neutral pH values (packing plant wastes, pH 6.8 and Sherwood Park raw, pH 7.6). On the other hand, the city and mixed raw samples, which have been limed, have pH values of 10.6 and 10.4 respectively. The temperature measurements show that the wastes leaving the packinghouses during the day are typically near 32°C. These are cooled slightly by the addition of the Beverly and Oliver wastes and by the flow to the lagoons, resulting in the 26°C temperature observed for the city raw. After the addition of the Sherwood Park wastes, at 13°C, the mixed raw enters cell 2 at about 23°C. The estimated flow data indicates that the city raw wastes account for 75% of the mixed raw. The calculated proportion of city raw in the mixed raw, based on the temperature data, is 77%, which agrees well with the flow estimations. The BOD, solids, greases and total carbon data show that the heavy organic load comes from the packing plants, and that the domestic wastes (as exemplified by the Sherwood Park raw) are very light by comparison. As an example, the BOD and grease levels in the Sherwood Park wastes are only 12% and 5%,

Table 10. Typical chemical and physical analyses of the Cloverbar lagoon influents and effluents

Sample	pH	Temp °C	mg/l BOD	Total Solids	Volatile Solids	Suspended Solids	Grease	Total Phosphate	Ammonia Nitrogen	Total Nitrogen	Inorganic Carbon	Total Carbon	C:N
Packing plant wastes[a]	6.8	32	2120	3360	1620	1850	1240	31	16	78	2	1060	13.6
City raw[b]	10.6	26	1720	3530	1310	1750	913	30	22	105	48	900	8.5
Sherwood Park raw[c]	7.6	13	181	808	396	188	64	26	26	43	30	195	4.5
Mixed raw[d]	10.4	23	1120	1940	1090	1170	785	31	28	89	30	450	5.0
Cell 2 effluent[e]	6.8	20	49	965	214	304	21	26	37	47	80	184	3.9
Cell 3 effluent[f]	6.9	21	59	870	153	134	6	21	41	47	87	218	3.4

[a]Sampled 1330 Thursday, November 8, 1973.

[b]Sampled 1500 Thursday, November 8, 1973.

[c]Sampled 1530 Thursday, November 8, 1973.

[d]Sampled 1500 Thursday, November 8, 1973.

[e]Sampled 0915 Monday, July 23, 1973.

[f]Sampled 0930 Monday, July 23, 1973.

respectively, of those in the packinghouse wastes. The nitrogen analyses show that the majority of this element present in the influent is in an organic form. The C:N ratios reported throughout this thesis, unless otherwise stated, are calculated using the total carbon and total nitrogen concentrations.

The lower portion of Table 10 summarizes the physical and chemical analyses of the effluents of cells 2 and 3. The pH values of these have dropped from the high values of the mixed raw to values near neutrality as a result of organic acid formation by the non-methanogenic bacteria. The temperatures of the lagoon effluents during the summer are near 20°C. However during the winter, the cell 2 effluent drops to about 17°C and the cell 3 effluent falls to as low as 13°C (Given et al., 1974). The large decrease in grease and suspended solids through the lagoons is due, to a large extent, to physical separation or stratification. The largest reduction of these parameters occurs in cell 2. The majority of the nitrogen in the lagoon effluents is in the ammonia form. Data in Table 10 indicate that the total nitrogen concentration leaving the lagoons is lower than that observed in the mixed raw. However, other data gathered during this study show that these levels remain fairly constant as the wastes pass through the system. As would be expected, the effluents have a much higher proportion of inorganic carbon than do the influents.

The physical and chemical data obtained for the strata of all three lagoons are summarized in Table 11. The samples were taken in mid October, 1973, at which time cell 1 was receiving one half of the Sherwood Park wastes treated with lime. These data, and those in Table 9, show that the pH values of the scum blankets are slightly lower that those at the 12 foot depth. The majority of the nitrogen in the scum and sludges is in an organic form, whereas, the middle samples of cells 1 and 2, show that there are nearly equal quantities of organic and inorganic nitrogen. However, ammonia nitrogen is the predominate form in the middle and surface samples of cell 3. No nitrate nor nitrite analyses were carried out since these are quickly reduced to ammonia under anaerobic conditions and are rarely found in such environments (APHA, 1971). Preliminary work showed that phosphate was not the limiting nutrient, so no further analyses were performed.

The high amounts of inorganic carbon found in the sludge samples are probably due to the accumulation of insoluble carbonates, mainly the calcium salt (from the addition of CaO). These are not readily available for bacterial assimilation, but provide much needed buffering capacity. Two different C:N ratios for each sample are given in Table 11. The first was derived using the concentration of total carbon present, while the second was calculated using only the organic carbon concentrations. (The total

Table 11. Chemical and physical analyses of composite samples of the Cloverbar lagoon strata

Sample		pH	mg/l								
			Total Solids	Volatile Solids	Grease	Ammonia Nitrogen	Total Nitrogen	Inorganic Carbon	Total Carbon	C:N	Organic C:N[a]
Cell 1[b]	Surface	6.7	147,000	117,000	86,000	163	2,280	300	73,600	32	32
	Middle	7.1	3,680	2,470	550	48	88	130	1,370	16	14
	Bottom	6.9	73,000	23,900	5,000	205	1,020	4,400	16,600	16	12
Cell 2[c]	Surface	7.4	119,000	98,200	90,000	223	770	100	31,300	41	40
	Middle	7.6	2,300	872	770	48	87	190	1,870	22	19
	Bottom	6.9	116,000	53,800	8,000	192	3,800	7,100	29,100	7.6	5.8
Cell 3[d]	Surface	7.1	916	222	24	48	56	93	213	3.9	2.1
	Middle	7.2	954	726	67	48	57	110	225	3.9	2.0
	Bottom	6.7	158,000	109,500	6,900	721	4,720	6,900	28,500	6.0	4.6

[a]Organic carbon divided by total nitrogen.

[b]Sampled Monday, October 15, 1973. One half of Sherwood Park waste were being treated in cell 1.

[c]Sampled Tuesday, October 16, 1973.

[d]Sampled Wednesday, October 17, 1973.

nitrogen concentrations were used in both cases.) These ratios differ only slightly, if at all, in the scum blankets on cells 1 and 2; by about 12% in the middle samples of the first two lagoons; by 25% in the sludges; and by 50% in the surface and middle samples of cell 3. Table 11 shows that the C:N ratios throughout the system deviate from the hypothesized ideal value of 10:1. Both cell 1, which has received raw wastes, and cell 2, which is receiving raw wastes, are deficient in nitrogen from the surface to at least the 12 foot depth. Lagoon 3, on the other hand, is deficient in carbon.

B. Recycling as a Means of Altering the C:N Ratio in the Liquid Portions of Cells 2 and 3

In order to increase the treatment efficiency of the Cloverbar lagoons, the C:N ratios should be adjusted to near 10:1.

One method of overcoming the nitrogen deficiency in cells 1 and 2 is the addition of ammonia to the system, instead of lime, in order to maintain a neutral pH. In doing so, nitrogen would be added, thus balancing the C:N ratio. However, the accumulation of nitrates, in natural waters, is now becoming a problem of great concern, and the addition of ammonia to the Cloverbar lagoons could result in higher levels of ammonia passing through the system to the North

Saskatchewan River, where, under the aerobic conditions, it would be microbiologically oxidized to nitrate. Hence, this simple solution could have an undesirable side effect.

During the summer of 1974, an attempt was made to adjust the C:N ratios in the liquid portions of cells 2 and 3 by recycling part of the effluent from the third lagoon back into the second. Table 10 shows that the cell 3 effluent has a low C:N ratio (most of the nitrogen being in the easily assimilated ammonia form). It was therefore felt that part of this could be added to the second lagoon to decrease the C:N ratio of the middle section of the lagoon. (However, due to stratification within the lagoons, it would be difficult to alter the C:N ratios in the scum and sludges by this means.) By adding a large enough volume of this effluent, some of the carbonaceous matter from cell 2 would be washed into cell 3, thus increasing the C:N ratio in the latter while decreasing the amount of carbon in the former. With the average daily flow through the system estimated to be near six million imperial gallons, it was speculated that the desired changes could be attained by recycling one million gallons/day.

The effluents from the two lagoons were monitored to determine the effects of recycling. Table 12 summarizes the data gathered from the Thursday samples (characteristic of weekday flow) and Table 13 gives the results of the Sunday samples. The large standard deviations reported, are due to

Table 12. Physical and chemical analyses of effluent samples taken from cells 2 and 3, before and during recycling of final effluent. Thursday effluent samples[a]

Sample	mg/l ±2SD								
	Volatile Solids	Suspended Solids	Grease	BOD	Ammonia Nitrogen	Total Nitrogen	Inorganic Carbon	Total Carbon	C:N
Cell 2 effluent									
Before	425 ± 69	386 ± 65	58 ±21	234 ±89	68 ±14	86 ±15	111 ±34	314 ± 43	3.6 ±0.5
During	393 ±205	400 ±226	61 ±47	254 ±73	64 ±20	85 ±29	128 ±28	339 ±110	4.0 ±0.7
Cell 3 effluent									
Before	284 ±31	184 ± 82	19 ± 3.6	130 ±64	61 ±11	72 ±12	110 ± 4.4	248 ±15	3.4 ±0.4
During	267 ±99	131 ±113	16 ±11	137 ±85	69 ±17	81 ±19	130 ±10	252 ±82	3.1 ±0.6

[a]averages based on 4 sampling days before recycling and 4 sampling days during recycling.

Table 13. Physical and chemical analyses of effluent samples taken from cells 2 and 3, before and during recycling of final effluent. Sunday effluent samples[a]

Sample	Volatile Solids (mg/l ±2SD)	Suspended Solids (mg/l ±2SD)	Grease (mg/l ±2SD)	BOD (mg/l ±2SD)	Ammonia Nitrogen (mg/l ±2SD)	Total Nitrogen (mg/l ±2SD)	Inorganic Carbon (mg/l ±2SD)	Total Carbon (mg/l ±2SD)	C:N (±2SD)
Cell 2 effluent									
Before	278 ± 101	258 ± 62	20 ± 1.4	112 ± 7.1	55 ± 9.9	66 ± 9.9	124 ± 7.1	250 ± 9.9	3.8 ± 0.7
During	258 ± 57	201 ± 126	22 ± 17	149 ± 60	59 ± 7.0	70 ± 9.1	132 ± 9.1	248 ± 28	3.5 ± 0.5
Cell 3 effluent									
Before	239 ± 42	198 ± 137	8 ± 4.2	112 ± 58	66 ±18	75 ±20	130 ± 14	250 ± 11	3.3 ± 0.7
During	236 ± 40	132 ± 112	11 ± 4.1	125 ± 29	74 ± 4.3	88 ±18	138 ± 11	238 ± 54	2.7 ± 0.6

[a]averages based on 2 sampling days before recycling and 4 sampling days during recycling.

the variations in the system from week to week.

Neither the weekday nor the weekend data show that recycling had the desired effects on the system. There was no significant increase in the amount of suspended matter nor the amount of carbon flowing from cell 2 after recycling had started. Nor was the quality of the cell 3 effluent altered. Therefore, it would appear that the volume which had been recycled, was inadequate. Upon checking the capacity of the pump used to return the cell 3 effluent, it was found that only a maximum of 0.5 million gallons/day could be moved instead of the proposed one million gallons/day. It was also found that the average daily flow through the lagoons was over 6.5 million gallons not six million as previously estimated. Both of these conditions would reduce the magnitude of the effects of recycling.

The figure of one million gallons/day, to be recycled, was arbitrarily chosen. This amount would have given 1 volume of recycled cell 3 effluent for 6 volumes of raw influent. These experimental conditions were nearly reached during the weekends when 0.5 volumes of effluent were mixed with about 3.4 volumes of domestic sewage (i.e. 1 volume to 6.8 volumes), yet the Sunday afternoon effluent samples showed no change due to recycling. Therefore, future attempts to adjust the C:N ration by this means, should recycle between 1.5 and 2 million gallons/day. During this endeavor, the cell 2 and 3 effluents should be monitored

both before and during recycling. A deterioration in the quality of the final effluent, would imply that too large a volume is being recycled, because more carbonaceous material is being washed into cell 3 than it can effectively digest.

Also, recycling should not be carried out during the winter months because it will cause the system to cool continuously which would, of course, be detrimental to the entire process.

Recycling did, however, have some effects on the contents of lagoons 2 and 3. The strata of these cells were sampled one week before recycling was started and again five weeks later. The analyses of these samples are given in Table 14. As expected, there was no significant change in the surfaces nor in the bottoms of the two cells. But, there was a large change in the middle sample of cell 2 and a slight change in that of cell 3. Samples taken from the 12 foot depth of cell 2 at points B3 and B4 (see Figure 18) had very high solids contents before recycling. It would appear that mounds of solid material had accumulated to a height of about eight feet, in the region between to two influent pipes. Five weeks later, during recycling, these mounds were not found. Thus recycling improved mixing within this lagoon. At the same time, the sludge samples obtained at points B1 and B2, near the recycle influent pipe had much less solid matter than the previous samples. This again indicates improved mixing.

Table 14. Physical and chemical analyses of composite samples of cells 2 and 3 strata taken before and during recycling of final effluent

Sample			mg/l						C:N
			Total Solids	Volatile Solids	Grease	Ammonia Nitrogen	Total Nitrogen	Inorganic Carbon	Total Carbon	
Cell 2	Surface	Before	113,000	93,500	87,000	110	820	140	49,100	60
		During	131,000	108,000	90,700	53	990	130	57,300	58
	Middle	Before	69,500	27,300	4,160	78	1,300	1,650	13,900	10.7
		During	2,820	1,090	220	64	120	160	820	6.6
	Bottom	Before	171,000	60,500	9,220	150	3,250	3,680	32,000	9.8
		During	184,000	66,300	6,120	130	3,380	4,410	29,300	8.7
Cell 3	Surface	Before	1,080	230	20	67	79	120	250	3.2
		During	1,070	190	14	58	68	120	260	3.8
	Middle	Before	1,280	370	44	67	87	120	340	3.9
		During	2,320	750	91	59	110	110	610	5.6
	Bottom	Before	207,000	74,600	8,360	760	5,760	7,560	36,500	6.3
		During	200,000	66,700	7,760	810	5,690	3,350	45,600	8.0

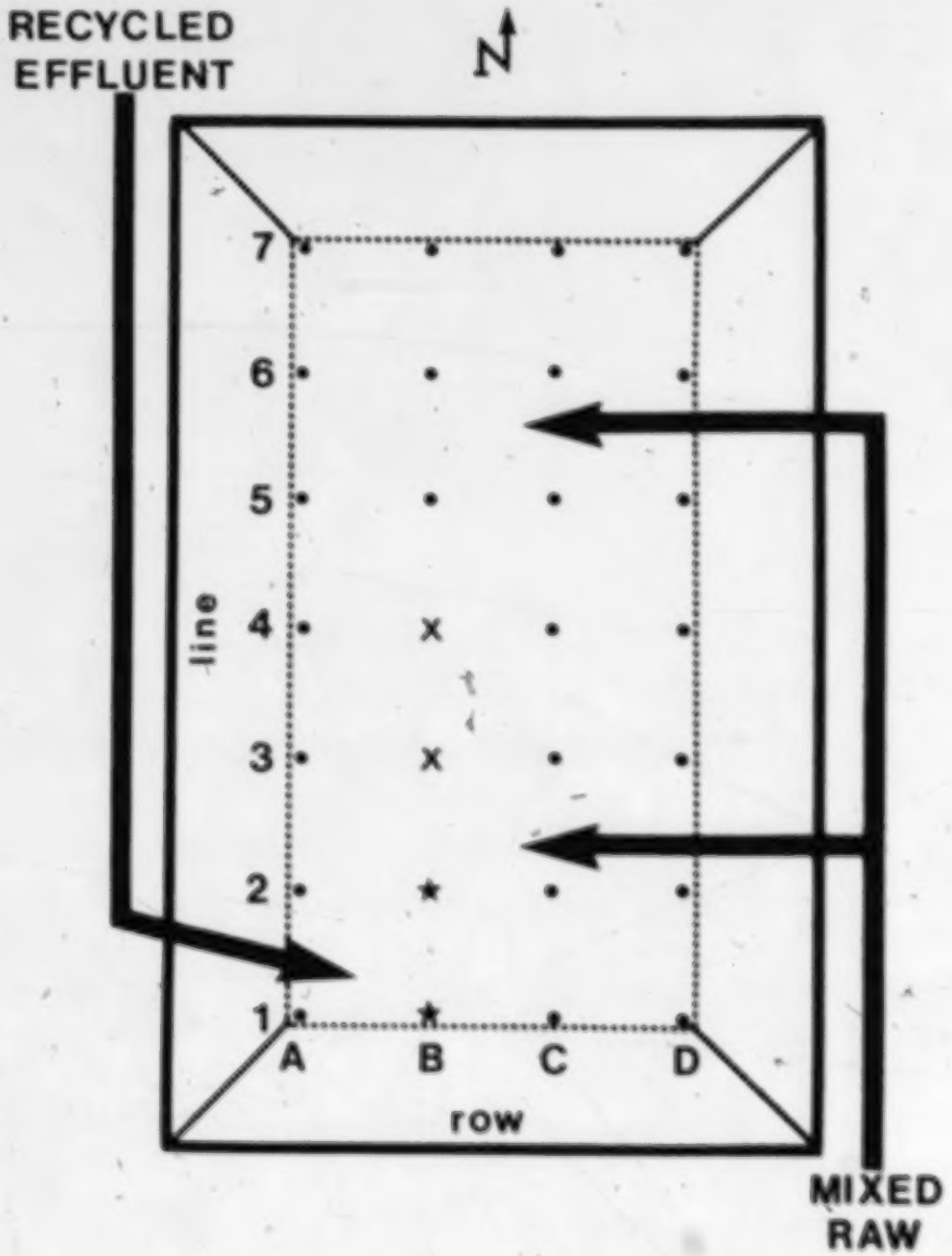


Figure 18. Locations within cell 2 where recycling caused marked changes in the nature of the samples taken from (X) the 12 foot depth and from (★) the bottom.

The composite sample from the middle of cell 3, during recycling, shows essentially a two fold increase in solids, grease and total carbon. This is difficult to explain since a corresponding change in these parameters was not observed in the cell 2 effluent. There is the possibility that the faster movement of wastes through the cell may have stirred up some of the sludge. However, no change in the quality of the final effluent was observed to support this hypothesis.

Although no attempt was made during this study, the nitrogen-rich cell 3 effluent could also be used to decrease the C:N ratio in the scum blanket on cell 1. Its surface could be flooded periodically with the effluent from cell 3 allowing the ammonia-rich liquid to percolate through the blanket. This should stimulate microbial activity and hopefully hasten grease digestion.

C. Increased Nitrogen Levels in the Cloverbar Industrial Lagoons while treating Sludge Supernatant

While collecting data prior to recyling, it was observed that the total nitrogen concentrations in the effluents of cells 2 and 3 were nearly double those found during the previous summer. Table 15 lists nitrogen levels in the cell 2 effluent samples taken from May, 1973 to October, 1974. These data show that the increase in total nitrogen is due mainly to the near doubling of the ammonia nitrogen concentrations. (Cell 3 effluent showed the identical trend.)

These increases corresponded to the period of time (between June 16 and October 2, 1974) when supernatant from the City of Edmonton sludge storage lagoons was treated by the Cloverbar industrial lagoons. However, the additional nitrogen decreased the C:N ratio only very slightly, if at all, indicating that carbon was also being added to the system. The October 17 sample shows that the nitrogen levels dropped back to their original values within two weeks after the completion of supernatant pumping.

Table 15. Effects of sludge supernatant[a] on the nitrogen concentrations in cell 2 effluent[b] - Clover Bar Lagoons

Sample date	Receiving Supernatant	Total Nitrogen	Ammonia Nitrogen	Organic Nitrogen	C:N
		mg/l			
May 14, 1973	No	45	33	13	4.3
July 23, 1973	No	47	37	10	3.9
May 29, 1974	No	43	29	14	4.5
July 31, 1974	Yes	77	59	18	3.8
August 19, 1974	Yes	77	63	14	3.4
September 11, 1974	Yes	87	69	18	3.8
October 17, 1974	No	39	30	9	4.6

[a] from the City of Edmonton sludge storage Lagoons

[b] identical trend observed in cell 3 effluent

D. Fatty Acid Analyses of Wastes from the Cloverbar Industrial Lagoons

Studies of the long chain fatty acids in the influent and the scum of cell 1 have been reported by Given et al. (1974). They found that 52% of the fatty acids in the packinghouse wastes were saturated (27% hexadecanoic and 23% octadecanoic acids) and near 45% unsaturated (37 to 44% cis-9-octadecenoic and 3% cis,cis-9,12-octadecadienoic acids).

The data gathered during this study differ only slightly from those of Given et al. (1974). The packing plant effluents analyzed have averaged 52% unsaturates, with the majority of this being cis-9-octadecenoic acid (42% of total fatty acids). Hexadecanoic and octadecanoic acids made up 27% and 17% of the total fatty acids respectively. A summary of typical fatty acid analyses of the influents and effluents (Table 16) shows that the predominate fatty acids throughout the system are cis-9-octadecenoic, octadecanoic, and hexadecanoic acids. The packing plant wastes differ from the Sherwood Park (domestic) wastes with respect to the abundance of these acids. A comparison shows that the domestic wastes contain higher proportions of hexadecanoic and octadecanoic acids (33% versus 25% and 27% versus 15% respectively), while the packing plant wastes have a greater abundance of cis-9-octadecenoic acid (44% compared to 28%). As the wastes pass through the two lagoons in series, the

Table 16. Typical long chain fatty acid analyses of influent and effluent samples from the Cloverbar lagoons

Sample	mg/l		Percent of total fatty acids						
	Grease	Total Fatty Acids	C12	C14	C16	C16:1	C18	C18:1	C18:2
Packing plant wastes[a]	1240	750	0.059	2.5	25	5.2	15	44	8.3
City raw[b]	910	500	0.084	2.6	25	5.2	15	43	8.2
Sherwood Park raw[c]	64	34	1.00	3.6	33	4.8	27	28	3.3
Mixed raw[d]	780	440	0.089	2.5	26	3.9	17	44	5.5
Cell 2 effluent[e]	49	16	0.13	2.2	33	1.8	28	33	1.5
Cell 3 effluent[f]	21	9.4	0.13	2.3	33	4.6	22	35	0.27

[a]Sampled 1330 Thursday, November 8, 1973.
[b]Sampled 1500 Thursday, November 8, 1973.
[c]Sampled 1530 Thursday, November 8, 1973.
[d]Sampled 1500 Thursday, November 8, 1973.
[e]Sampled 0930 Friday, May 31, 1974.
[f]Sampled 1500 Thursday, September 5, 1974.

proportion of cis-9-octadecenoic acid decreases slightly and the proportions of hexadecanoic and octadecanoic acids increase slightly. Viswanathan et al. (1962) noted that cis-9-octadecenoic acid was degraded much more rapidly than any of the saturated fatty acids. This "degradation" probably involves its biohydrogenation to octadecanoic acid. The extent to which biohydrogenation occurs through the Cloverbar lagoon system was estimated by analyzing four samples, taken at different times, from each of the following locations: mixed raw influent, cell 2 effluent, and cell 3 effluent. The average percentages of unsaturates (±1 standard deviation) were found to be 49 (±6), 37 (±2), and 37 (±5), respectively. Therefore, the majority of the biohydrogenation observed, occurred in cell 2.

Also of interest, is the comparison of changes in fatty acid proportions occurring within the lagoons(Table 17). The most noticable changes are the decrease in the proportion of cis-9-octadecenoic acid and the increase in proportion of hexadecanoic acid in cells 1 and 2. It would seem most probable that the decrease in the abundance of cis-9-octadecenoic acid (due to biohydrogenation) would lead to an increase in the relative amount of octadecanoic acid. However, this is not observed. Instead, there is an accumulation of hexadecanoic acid. This occurrence could be explained by one of two mechanisms. Firstly, the octadecanoic acid which is formed may be degraded by a

Table 17. Long chain fatty acid analyses of composite samples of the Cloverbar lagoon strata

Samples		mg/l		Percent of total fatty acids						
		Grease	Total Fatty Acid	C12	C14	C16	C16:1	C18	C18:1	C18:2
Cell 1[a]	Surface	86,800	68,200	0.054	2.3	65	-[d]	23	9.2[e]	-
	Middle	500	400	0.085	2.7	62	-	29	6.2	-
	Bottom	5,000	2,500	0.13	2.1	62	-	30	6.1	0.08
Cell 2[b]	Surface	90,000	68,800	0.06	3.3	54	1.4	25	16[d]	0.15
	Middle	770	635	0.02	3.2	51	6.6	29	11	-
	Bottom	8,000	2,600	0.18	3.0	42	2.5	28	24	0.79
Cell 3[c]	Surface	24	7	-	12	33	4.9	22	26	0.79
	Middle	67	17	1.8	2.9	45	3.3	26	21	-
	Bottom	6,900	660	-	15	40	5.6	28	12	0.076

[a]Sampled Monday, October 15, 1973. One-half of Sherwood Park wastes were being treated in Cell 1.

[b]Sampled Tuesday, October 16, 1973.

[c]Sampled Wednesday, October 17, 1973.

[d]None detectable.

[e]Present in inhibitory concentrations.

beta-oxidation, giving hexadecanoic acid which accumulates; or secondly, the former may be preferentially used by the non-methanogenic bacteria, resulting in a negative enrichment of the latter.

Figure 19 compares the relative amounts of selected fatty acids present in the mixed raw with those in the scum blankets in the first two lagoons. Figure 19(a) clearly shows the effects of biohydrogenation. The percentage of unsaturates falls from near 50% in the raw influent to values of about 10% in the cell 1 scum and 18% in the cell 2 scum. Figures 19(b) and (c) show that the relative abundance of hexadecanoic acid has greatly increased in the grease blankets, while the proportions of octadecanoic acid has remained fairly constant.

Galbraith et al. (1971) have studied the antibacterial activity of long chain fatty acids and have shown that the unsaturated acids are more toxic than the corresponding saturated acids. Therefore, biohydrogenation serves to detoxify these compounds. Prins et al. (1972) reported that a concentration of 10^{-2} M (2820 mg/l) cis-9-octadecenoic acid inhibits methane formation by about 85%. As shown in Table 17, both scum blankets have concentration well above this value. Thus, it is doubtful that any methane formation can occur in the scum. These high levels are not found elsewhere in the system.

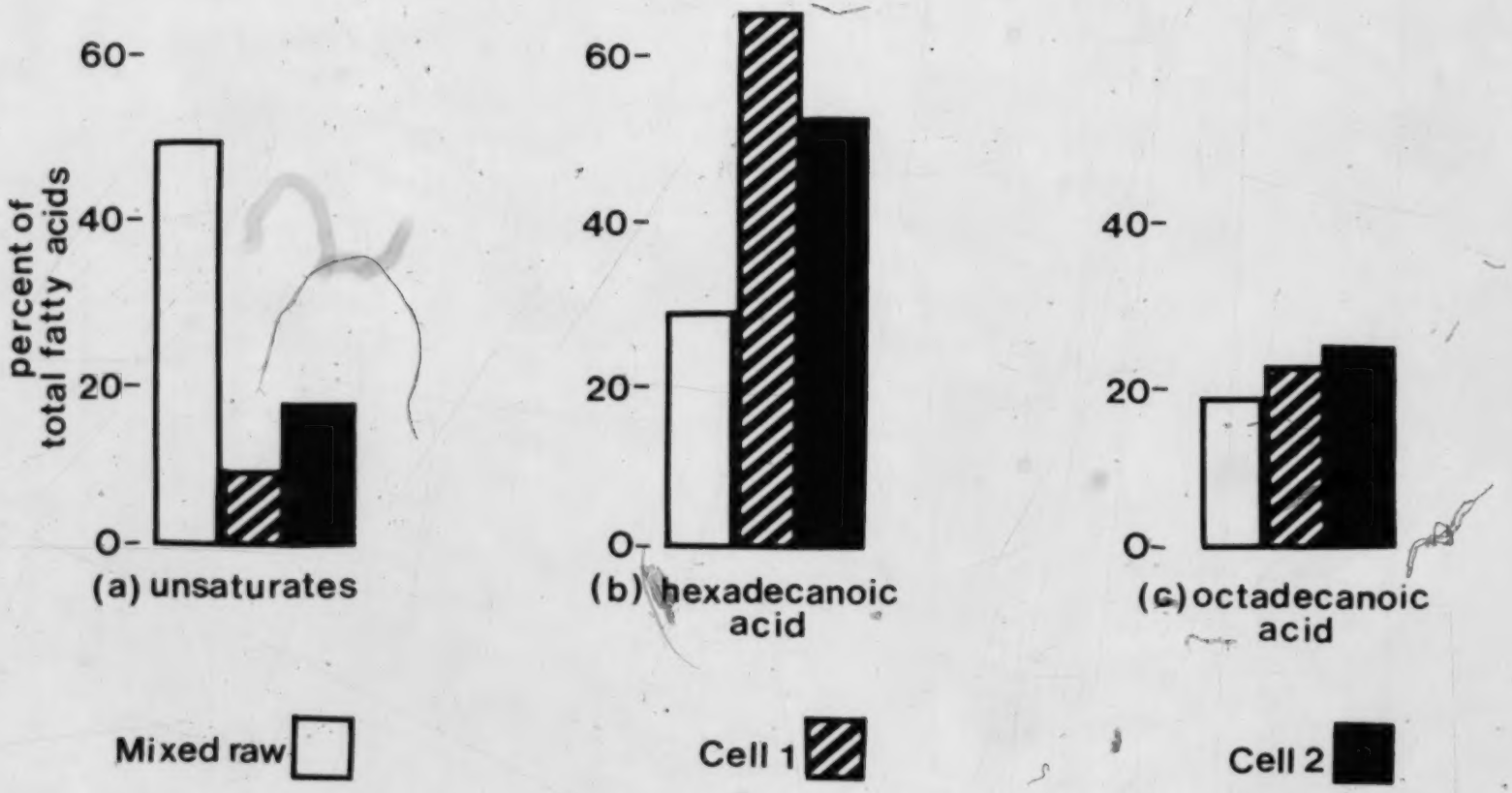


Figure 19. A comparison of selected long chain fatty acids in the scum blankets and in the mixed raw influent.

The grease and fatty acid analyses of sludge samples from the three lagoons show that each one is unique. Figure 20(a) shows that the grease contents of cells 2 and 3 are nearly the same, with the cell 1 sludge containing a slightly lesser amount of grease. Cell 1 has 5,000 mg/l, cell 2 has 8,000 mg/l and cell 3 has 6,900 mg/l. However, the fractions of long chain fatty acids within the sludges do vary as illustrated in Figure 20(b). Cells 1 and 2, which have both received raw wastes, have fairly high fatty acid concentrations, 50 and 32% of the total grease, respectively. By comparison, cell 3, which has never received raw wastewaters, has sludge containing only 9.5% fatty acids.

Figure 20(c) shows that the sludges also vary in their proportions of unsaturated fatty acids. Cell 1 has the lowest relative amounts of unsaturates (6.2%) whereas cell 2 has the highest amounts (27%). These observations might be explained in terms of sludge age. That is, the sludge in the first lagoon has been in the system longer than the sludge is cell 2, therefore, it has undergone biohydrogenation to a greater extent. Also, for some time prior to the beginning of this project, cell 1 had not been receiving any high strength wastes. These flowed directly into cell 2. Thus, the latter was receiving a fresh supply of unsaturates, which would obviously retain higher relative levels of these acids even if biohydrogenation was occurring at the same

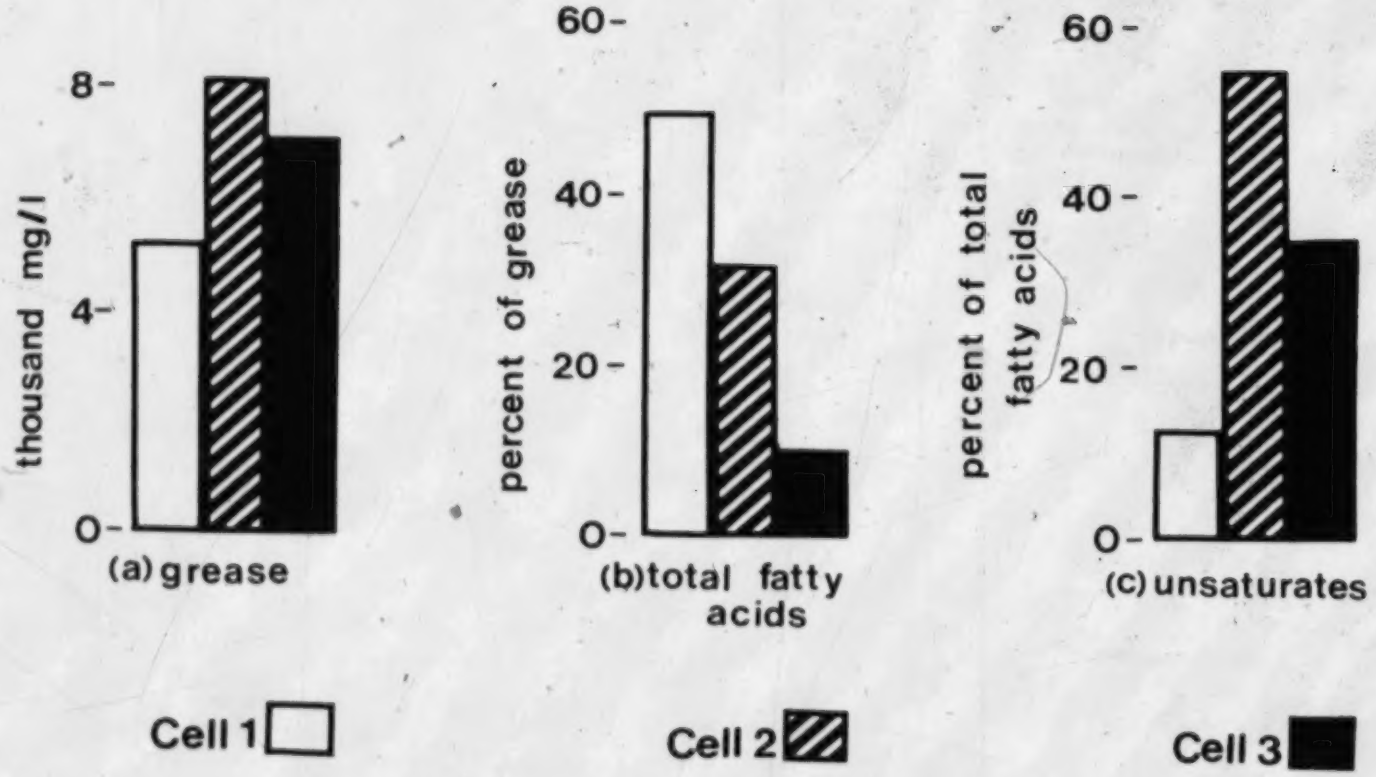


Figure 20. A comparison of grease and fatty acid composition of sludges in the three anaerobic lagoons.

rate in both lagoons.

SUMMARY AND CONCLUSIONS

This investigation has considered the effects of dilution methods on viable bacterial counts in sewage samples; the numbers of bacteria throughout the Cloverbar industrial lagoon system, with particular reference to the sulfate reducers; and the physical and chemical characteristics of the wastes as they pass through the lagoons, considering mainly the C:N ratios and the nature and quantities of the long chain fatty acids present.

The finding from these studies are listed below:

1. The numbers of viable sulfate reducers detected in a sewage sample with a neutral pH, is nearly independent of the extent to which the dilution buffer was prereduced.

2. Alkaline wastewater samples, which are collected for bacterial counts, should be diluted as soon as they are taken in order to reduce excessive loss of viability due to the high pH during transit.

3. Lime addition in the city, giving a pH of 11.8, does not significantly decrease the numbers of sulfate-reducing bacteria in the sewers. Lime, therefore, should only be added to maintain a pH of 9 in the sewage lines, in order to chemically trap

the hydrogen sulfide present.

4. During the winter, lime should be added at the lagoons, to maintain cells 2 and 3 between pH 7.0 and 7.2 to ensure the survival and activity of the methane-producing population. During the summer, cell 2 should be maintained near pH 7.5 to increase grease digestion, while cell 3 is maintained between pH 7.0 and 7.2 to maximize methane production.

5. The presence of large numbers of sulfate reducers at the surface of all three lagoons, indicates that anaerobic conditions exist throughout each lagoon.

6. A scum blanket with a large population of sulfate reducers and a pH below about 7.2, will produce hydrogen sulfide rather than control odors from the lagoon which it covers.

7. Since a neutral pH is very important to the maintance of an active methane fermentation, elevated pH levels should not be used in attempts to control hydrogen sulfide odors from the lagoons. This gas may be trapped as FeS by the addition of scrap iron.

8. Chemical analyses show that the C:N ratios, throughout the system, deviate from the 10:1 value

which is theoretically ideal for maximum bacterial activity.

9. The C:N ratios in cells 2 and 3 might be adjusted by recycling a fraction of the nitrogen-rich final effluent into cell 2. With the daily influent flow rate of just over 6.5 million gallons, the desired results might be attained by recycling between 1.5 and 2 million gallons/day.

10. Flooding of the surface of cell 1, periodically with the final effluent, may decrease the C:N ratio in the grease blanket to a level which is more suitable for microbial activity, hence, hastening the breakdown of the scum.

11. Recycling appears to improve the mixing within cell 2.

12. The ammonia-nitrogen concentations in the effluents, are nearly doubled when the Cloverbar lagoons receive the sludge supernatant from the sludge storage lagoons.

13. Biohydrogenation in the Cloverbar lagoons, occurs mainly in cell 2.

14. In the grease blankets, there is a large decrease in the proportion of cis-9-octadecenoic acid, and an accumulation of hexadecanoic acid.

15. The concentration of cis-9-octadecenoic acid in the scum layers is at a level which is inhibitory to methane bacteria.

16. The sludge in cell 3 differs markedly from the sludges in the other two lagoons with respect to fatty acid composition.

SUGGESTIONS FOR FURTHER STUDIES

The experience gained during this project would suggest the need for further study in the following areas:

1. The "plot" experiments should be repeated using a control, the three treatments previously described, and at least two other treatments. One of these new treatments should be the periodic addition of several gallons of cell 3 effluent to the plot surface allowing the liquid to perculate through the grease. The second treatment should be a combination of urea and cell 3 effluent which would distribute this nitrogen source through the crust.

2. Recycling part of the cell 3 effluent should again be attempted during the summer. However, this time a larger volume (between 1.5 and 2 million imperial gallons/day) should be recirculated in order to achieve the desired results. The cell 2 and 3 effluents should be monitored both before and during recycling and if the quality of the latter declines, the volume being pumped should be reduced. This process must establish optimal conditions of mixing and stratification resulting in the maximum treatment efficiency.

3. The efficiency of scrap iron to trap hydrogen sulfide should be tested in the field. Such a test may be carried out on the surface of cell 1 (which contains large numbers of sulfate reducers) using "gas-tight plots" equipped with a fermentation lock containing a solution of lead acetate to trap the hydrogen sulfide which was liberated. This device would allow a comparison of the amounts of hydrogen sulfide escaping from a control plot and a test plot.

4. Laboratory studies using radioactive fatty acids should be undertaken to determine why there is an accumulation of hexadecanoic acid in the scum blanket. The evolution of labelled methane or carbon dioxide from 3-^{14}C-cis-9-octadecenoic acid would indicate that after biohydrogenation, this compound undergoes at least two beta-oxidations. This would imply that the increased proportion of hexadecanoic acid observed in the scum, was due to negative enrichment. If, on the other hand, 1-^{14}C-hexadecanoic acid accumulates, it would be a result of biohydrogenation followed by only one beta-oxidation meaning that the microbes involved in these conversions would metabolize octadecanoic acid more readily than hexadecanoic acid, leaving the latter in the medium to accumulate.

5. The final effluent from the lagoons produces a foam as it enters the North Saskatchewan River. This is probably due to detergents which pass through the anaerobic process with little of no degradation. This situation may be improved, during the summer months, by using the storage lagoons as aerobic treatment ponds. The removal of detergents by this oxidative process could be evaluated by monitoring their levels in the cell 3 and final effluents.

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APPENDIX

Modified Butlin's Broth[a]

K_2HPO_4	0.5g
NH_4Cl	1.0g
Na_2SO_4	2.0g
$CaCl_2 6H_2O$	0.1g
$MgSO_4\ 7H_2O$	1.0g
Sodium Lactate (60% solution)	2.5 ml
Yeast Extract	1.0g
$FeSO_4$	0.002g
Water	1000 ml

Adjust to pH 7.5

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[a]After Butlin, K.R., Adams, M.E., and Thomas, M. 1949. The Isolation and cultivation of Sulfate Reducing Bacteria. J. Gen. Microbiol. 3:46-59.